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STUDIES IN PLAGUE IMMUNITY.[1]

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I. INTRODUCTION.

The experimental work which constitutes the basis of this report was undertaken with the object of throwing further light upon certain practical problems in relation to pest immunity, and particularly with the idea of determining the most efficacious method of protective inoculation against plague. The subject of immunization against pest is not only of general scientific interest, but at least to several tropical and subtropical countries is of great practical importance. One need only recall the mortality in India of nearly a million deaths from this disease during the past year (1905) to be impressed with the importance of the problem, and while it is true that at present no epidemic of plague exists in the Philippine Islands, yet sporadic cases occur from time to time and it must be recalled that only a few years ago (1903) it was thought by the Board of Health advisable and necessary to perform among the Chinese of the city of Manila general inoculations against this disease.

It is true that the subject of protective inoculation against plague has received considerable attention during the past few years and that prophylactics have been recommended by several authors, but, while it is admitted that by their use a certain degree of pest immunity can be produced and demonstrated in a number of the more insusceptible animals and, occasionally, even in those very susceptible to this infection, nevertheless, it has sometimes seemed questionable whether we were able, by the inocultion of these prophylactics, to obtain in man an immunity of such a degree as to be protective against the natural and usual methods of infection by the malady. Thus, Kolle called attention to the fact that since, by the use of even very large doses of the killed pest organisms only exceptionaly were we able to immunize against pest infection the animal which is most susceptible to plague, namely, the guinea pig, it seemed very doubtful if favorable results could be obtained in man, where relatively much smaller doses of the killed cultures were injected. Yet, from 1898, when protective inoculation was first introduced, up to the time of my first report on vaccination against plague in November, 1905, no other methods except those in which the killed cultures were employed had been used in the very extensive human inoculations performed against this disease in India and in other countries.

In the latter part of the year 1903, when the Board of Health of Manila was practicing among the Chinese in this city protective inoculation against plague by the injection of the killed cultures of the pest bacillus, the method at that time carried on in Japan and consisting of

the injection of 1 oese of a 24-hour slant agar culture of *Bacillus pestis* suspended and killed in 1 cubic centimeter of 0.085 saline solution, I decided to investigate whether any immune substances became developed in the serum of the inoculated individuals. It did not seem possible to me that any appreciable degree of immunity could be acquired from these inoculations, owing to the small size of the dose and the very mild local and general reaction which resulted from the injections. I therefore studied the agglutinative and bactericidal reactions of the blood serum of 12 cases, 6 of whom had been inoculated two weeks and 6 three weeks previously with 1 oese of the killed pest culture. The agglutinative reactions were performed by the macroscopic method and the bactericidal reactions according to the one suggested by Neisser and Wechsberg. No traces of agglutinins or of bacteriolysins could be demonstrated in the sera of any of the individuals.[2] Obviously, these experiments in themselves were not considered to be conclusive evidence of the fact that no immunity was conferred upon the inoculated, since it was already recognized at this time that these antibodies were frequently and indeed, usually, not encountered even in the blood sera of individuals who had recovered from an attack of plague and were immune to this disease.[3] Therefore, experiments in animals were resorted to in order that more information on this subject might be obtained. Ten guinea pigs were inoculated subcutaneously, each with the same dose that was being employed in the general human inoculations in this city. After two weeks the immunity of these animals was tested in the following manner. One oese of a virulent pest organism was suspended in 1 cubic centimeter of 0.085 saline solution and 5 oesen of this suspension rubbed over a freshly shaved area on the abdomen of the guinea pig. All of the animals succumbed to acute pest infection, demonstrating conclusively that an immunity of appreciable degree had not been produced. A short time after, the important paper of Kolle and Otto[4] was published in which the unfavorable results from the immunization of guinea pigs with large doses of killed agar cultures of the pest bacillus or with Haffkine's prophylactic were reported. It therefore seemed to me, at that time, more advisable to experiment further with other methods of immunization against plague, before insisting upon the use of larger amounts of more virulent

[2] These experiments were undertaken at this time because the statement had previously been made that agglutinins, at least in some cases, had been demonstrated in the blood serum of human beings who had been inoculated against plague a short time before with Haffkine's prophylactic, a conclusion which I have not been able in any manner to confirm.

[3] Experiments demonstrating the fact that animals immune to pest infection may still show no traces of agglutinins in their blood, together with those demonstrating the absence of a true bacteriolytic action of plague immune serum, will be presented later in this paper.

[4] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 507.

killed pest cultures in the human inoculations being pursued in this city. Further results obtained by Kolle and his associates in Berlin, and the animal experiments made here, having demonstrated that in very susceptible animals at least, satisfactory immunization could only exceptionally be produced by the methods of human inoculation then in vogue, this laboratory recommended to the Commissioner of Health in Manila the suspension of the human inoculations with killed cultures of the pest bacillus, and has not since recommended to our Government any method of protective inoculation against plague as an efficient procedure against the extension of this disease. However, further experimentation, which I have carried on as continuously as was practicable for the past year and a half, has demonstrated that a satisfactory immunity can be obtained in the guinea pig, *an animal even more susceptible to plague infection than is man*, by the inoculation of living cultures of plague bacilli of such attenuation that they are no longer dangerous when injected into human beings. Therefore, this laboratory believes that by the method of *vaccination*[5] against plague with suitable cultures, we have probably an efficient measure for the control of this infectious disease, and we recommend its adoption for this purpose.

At present there is not sufficient plague in this city to warrant the employment of general vaccination against the malady, but it is hoped that the method may be given a thorough and careful trial in certain districts of India where plague is always present in sufficient amounts to justify its use. A preliminary report on the subject of vaccination against plague was presented to the Manila Medical Society in November, 1905, and published in an earlier number of this JOURNAL.[6] In the present article further evidence of the entire safety of vaccination in man against plague with suitable cultures will be presented, together with the experimental work which demonstrates conclusively the efficacy of this procedure and its superiority to other methods of protective inoculation, including those in which both natural and artificial plague aggressin, so recently described, are employed.

[5] The term "vaccination" is employed in this article only in the sense in which it was primarily used by Jenner and Pasteur (immunization with the living, attenuated organism) and is not applied to forms of protective inoculation in which the killed organisms or their extracts are employed. See Kolle's previous remarks on this subject.

[6] *This Journal* (1906), 1, 181.

II. DISCUSSION OF THE PROPHYLACTICS PREVIOUSLY EMPLOYED.

Before proceeding directly to this discussion of the experimental work, it will be advantageous to mention the various prophylactics which have already been used in human beings, and briefly to review the methods of preparing them and the immunizing powers which they possess.

1. METHODS OF PREPARATION.

Haffkine's[7] prophylactic against plague consists of the killed bouillon cultures of the pest bacillus. The organism is grown in 3-liter flasks of bouillon upon the surface of which clarified butter or coconut oil is distributed. The cultures are kept for six weeks at a temperature between 25° and 30° C. The bacteria form stalactite-like growths, which extend from beneath the surface of the oil downwards. The flasks are shaken every few days, when the organisms fall to the bottom of the vessel and a new growth takes place above. After five or six weeks' growth, in order to be sure that the bouillon cultures are pure, subcultures are made from them upon agar slants. The bouillon cultures are then killed by heating for one hour at 65° to 70° C. and, after their sterility is proven, sufficient carbolic acid is added to form a 0.5 per cent solution. The prophylactic is placed in 30 cubic centimeter bottles and it must be shaken before use, since the killed bacteria naturally settle to the bottom of the vessel. The dose for men is from 3 to 3.5 cubic centimeters, for women from 2 to 2.5 cubic centimeters and for children from 0.5 to 1 cubic centimeter. Recently, Haffkine has in special cases recommended as high as 20 cubic centimeters for a single injection. He also frequently advises a second and even a third inoculation.

Another form of prophylactic against plague is that which was recommended by the German Plague Commission[8] (Gaffky, Pfeiffer, Sticker and Dieudonné) and which consists of the killed agar cultures of the virulent pest bacillus. Forty-eight hour agar slants of the organism are suspended in saline solution or bouillon and killed by heating for one hour at 65° C. Carbolic acid is then added to 0.5 per cent. The dose for a grown man is one agar culture. This prophylactic has many advantages over that recommended by Haffkine since with it an accurate dose as well a fixed virulence for the organism employed can be obtained. Moreover, it is much less dangerous, since either the tetanus bacillus or the organism of malignant œdema may develop in the bouillon cultures. Such anaërobic organisms will naturally not multiply on the agar slants.

Haffkine claimed as an advantage of his method that certain metabolic substances of the plague bacillus were contained in the fluid media which rendered the prophylactic more effective.

[7] *Brit. Med. Journ.* (1897), 1461; also Lancet (1899), **77**, 1695.

[8] Bericht. der deutsch. Pest Kommission. *Arb. a. d. k. Gsndhtsamte*, Berl. (1899), **16**, 306.

However, both the German Plague Commission and Kolle have shown that the immunizing substances in Haffkine's prophylactic are mainly contained in the sediment consisting of the killed organisms. Kolle[9] showed that the clear fluid drawn off above the dead bacteria possessed but little immunizing power and further demonstrated that the number of bacteria in the old bouillon cultures was very small in proportion to the number that could be obtained in the same volume of fluid if agar cultures were used. Thus, one agar culture contained about as many bacteria as from 80 to 100 cubic centimeters of an old bouillon culture. Obviously whatever immunizing power the clear fluid above the sediment possesses is due to the free receptors which have been separated from the dead bacilli. It therefore seems evident that killed agar cultures are superior for inoculation to the killed bouillon ones.

Lustig and Galeotti in 1897[10] recommended the use of an extract of the plague bacillus obtained by chemical reagents. For its preparation three or four day agar cultures of the organism are thoroughly shaken with 1 per cent caustic potash solution.

After two hours, a 0.5 per cent acetic acid solution is slowly added until the mixture becomes slightly acid, when a flocculent precipitate forms. This sediment is collected on filter paper, washed to a neutral reaction and finally dried in vacuo. The powder when it is to be used is dissolved in 1 per cent sodium carbonate solution. The dose for man is from 2 to 3 milligrams. The Swiss serum institute in Bern recommends this prophylactic particularly because of the fact that an exact dose can be obtained and on account of the ease with which it can be transported.

Another protective against plague has been recommended by Terni and Bandi[11] in 1900. For the preparation of this prophylactic guinea pigs or rabbits are inoculated intraperitoneally with a small quantity of a suspension of the pest bacillus in bouillon. The animal usually dies in from thirty-six to forty-eight hours. At the time of its death, or a little before (in order to be sure that no migration into the peritoneal cavity of the intestinal bacteria has taken place) the abdominal cavity is opened, the peritoneal exudate collected and, if it is very thick, diluted with saline solution. The exudate is then placed in the incubator at 37° C. for twelve hours; it is next heated at from 50° to 52° C. for one hour on two successive days; in this way it becomes sterilized and the serum albumins present are not coagulated. A mixture of carbolic acid, 0.5 per cent, sodium carbonate 0.25 per cent, and sodium chloride 0.75 per cent is then added, in order to aid in the preservation and in the absorbability of the prophylactic when used. From 1.5 to 2.5 cubic centimeters are recommended for human injections.

The method of inoculation against plague recommended by Shiga[12] is as follows: The growth from a three-day agar culture which contained about 3 oesen of plague organisms was placed in a mortar with 3 cubic centimeters of salt solution and thoroughly rubbed up. The suspension was then heated for 30 minutes at 60° C. and later, carbolic acid to 0.5 per cent was mixed with it. After twenty-four hours an equal volume of a pest immune serum was added. Shiga recommended two injections; for the first one, 0.6 to 1 cubic centimeter of the mixture of serum and organisms and for the second, after the

[9] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1901), **36**, 396.

[10] *Deutsche med. Wchnsch.* (1897), **23**, 227, 289.

[11] *Deutsche med. Wchnsch.* (1900), **26**, 463; also *Rev. d. Hyg.* Paris (1900), **22**, 62.

[12] Ber. über die Pest in Kobe und Osaka, Tokyo (1900), 54.

reaction had subsided, the same amount of the suspension of the killed organisms alone. He also advised a third inoculation with a larger dose for those who are frequently exposed to plague infection. The addition of the plague serum is recommended in order to diminish the local and general reaction and to aid in absorption.

The prophylactic recommended by Besredka[13] in 1902 is prepared by adding a plague immune serum to a suspension of the killed pest bacillus in saline solution. By this means the bacteria become agglutinated and sink to the bottom of the vessel. The organisms are killed by heating for one hour at 60° C. The precipitate of bacteria is then washed thoroughly to free it from any excess of serum, and the agglutinated and washed bacteria are used for the injection. This prophylactic, Besredka maintained, produced an immunity in animals after forty-eight hours, which lasted for five months. Recently[14] he has recommended the addition of a normal horse's serum in place of the immune serum, to the suspensions of the killed and dried bacteria in saline solution. The organisms after separation by centrifugation are then said to be "atoxique" and are used for the inoculations.

Gosio[15] recently has described in detail a practical method of preparing plague prophylactic in large quantities. Pest bacilli were cultivated in thin layers of bouillon, in flasks such as are used for the preparation of diphtheria toxin. The bacteria were then precipitated by means of an agglutinating pest serum and separated from the latter; following this they were killed by heating at 65° C. for one hour, and the sterility of the mixture proved by the addition to the inoculated bouillon tubes of a small amount of potassium telluride.

Hueppe and Kikuchi[16] in October, 1905, announced that successful results had been obtained in guinea pigs by repeated inoculations of exudates containing plague aggressin. The experiments are very few in number and but briefly related, but the authors mention that in this paper it is their intention merely to call attention to the priority of the use of this method of immunization of animals against plague. As yet they have not reported further upon the subject.

In November, 1905, I for the first time reported to the Manila Medical Society upon vaccination in man with attenuated living cultures of the pest bacillus, and later, in February, 1906, published in this JOURNAL[17] and afterwards with Professor Kolle,[18] further experiments relating to this subject. The details of all the work performed in vaccination with these cultures will be considered in the present paper.

Finally, in January of this year (1906) Klein[19] has described a method of plague inoculation which he has employed in white mice, rats and guinea pigs. The buboes, spleen, lungs and liver of a guinea pig which has succumbed to subacute pest infection were removed and finely minced aseptically, spread out in thin layers in sterile glass dishes and dried over sulphuric acid at a temperature

[13] *Ann. d. l'inst. Pasteur* (1902), **16**, 918.
[14] *Ann. d. l'inst. Pasteur* (1905), **19**, 479.
[15] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1905), **50**, 519.
[16] *Centrbl. f. Bakteriol. Orig.* (1905), **39**, 610.
[17] *This Journal* (1906), **1**, 181.
[18] *Deutsche Med. Wchnsch.* (1906), **32**, 413.
[19] "Preliminary Report to the Local Government Board on a New Plague Prophylactic," 1906, London, Darling and Son; also *Brit. Med. Journ.* (1906), 155.

of 46° to 47° C. for three days. The exposure at this temperature devitalized all the pest bacilli in the organs and also prevented the growth and multiplication of other extraneous organisms. After three days of desiccation the dried scales of the material were rubbed down to a fine powder in a sterile mortar. The material was finally dried for from two to three days, at 37° C., in a wide-mouthed bottle.

When the prophylactic was to be used the desired amount was weighed out, well rubbed down in sterile warm distilled water and the turbid emulsion thus obtained injected subcutaneously.

This material is said to contain not only "acutely active toxin but also the dead bodies of all the *Bacilli pestis* originally present in large numbers in the necrotic organs (buboes, spleen, liver and lungs) with the addition probably of other substances of an undetermined nature and action."

Klein states that 10 to 15 milligrams of the dried powder confers immunity on the adult rat whereas 5 cubic centimeters of Haffkine's prophylactic, strongly turbid with flakes and masses of bacilli, does not do so, 10 cubic centimeters being necessary. The above prophylactic, in doses of 1 to 5 milligrams, killed a large percentage of mice within twenty to twenty-four hours and from 12 to 25 per cent of half-grown white rats in doses of from 5 to 8 milligrams, if the material was derived from acute cases. However, if obtained from the necrotic organs of guinea pigs dead of subacute plague (death in from five to nine days) as much as 10 to 12 milligrams were required to produce a fatal effect. Even as large amounts as 20 milligrams failed to kill a guinea pig of 200 to 300 grams weight. An adult rat weighing 120 to 200 grams, injected with 10 to 15 milligrams of the prophylactic of medium virulence, or with two doses of 10 milligrams each at an interval of nine to ten days, was given complete protection against even the most virulent *Bacillus pestis* when tested from one to thirteen weeks after immunization. A dose of 20 milligrams of the prophylactic in question in the case of guinea pigs, (which animals Klein regards as *less* susceptible to plague than white rats) twice injected at intervals of ten to fourteen days did not afford protection in more than 50 per cent of the animals, the remainder dying upon the subsequent injection of virulent plague material. However, the death of the animals was delayed several days and in the great majority of instances, they showed suppurating buboes. Klein argues that 10 cubic centimeters of Haffkine's prophylactic will protect an adult rat, an amount which according to the statistics in India and elsewhere represents at least double that required for the protection of an adult human being, so that 5 to 7 milligrams of his dried prophylactic would suffice as the dose for the human subject.

S. Wallannah[20] has also described a somewhat similar method of obtaining an extract from plague organs he proposes for the treatment of plague cases, arguing that the lesions are probably the centers where the pest antibodies are manufactured in great quantity.

Passive immunization with serum Yersin, Calmette and Borrell[21] will be considered later in this article. While this method is particularly efficacious for a short period of time after the inoculation, the protection afforded by it persists only for a few days. Eight days after the injection its protective action becomes weaker, and after twelve it is almost entirely lost.

[20] *Centrbl. f. Bakteriol. Orig.* (1906), **42**, 471, and *Lancet* (1907), **172**, 222.
[21] *Ann. d. l'inst. Pasteur* (1895) **9**, 589.

2. CONSIDERATION OF THE EXTENT EMPLOYED IN MAN AND THE RESULTS OBTAINED.

The prophylactics described above constitute the methods of immunization against plague which have been recommended from the beginning of the nineteenth century up to the present time.[22] In regard to the extent to which they have been used in man it may be said that Besredka inoculated himself with his prophylactic but, so far as I am aware, it has not been further employed in human beings. The methods of Gosio, Hueppe and Kikuchi and Klein have not, to judge from the absence of reports, as yet been used in man. Forty-seven persons were inoculated by Shiga's method in 1899 in Kobe and Osaka. The Terni-Bandi method was employed in Rio de Janeiro, Brazil, in the epidemic of 1889 to 1901. According to Havelburg several hundred people were inoculated in that city and no cases of plague occurred among them, with one exception, where the individual sickened on the day of inoculation and where the attack of pest was mild and resulted in the recovery of the patient. The inhabitnats of Rio de Janeiro number about 750,000, and only 589 cases of plague occurred in the entire city; therefore from these human statistics, we can not form a judgment of the value of the method. Dessy in the plague epidemic in 1900, in San Nicola, La Plata, inoculated 200 persons with Lustig's prophylactic. None of the vaccinated sickened with pest. The same remark applies to these statistics as to the ones collected from the cases in which the Terni-Bandi method was employed. The prophylactic recommended by the German Plague Commission has also not been very extensively employed in human beings, although Zupitza has used the method to some extent in East Africa. Over 127,000 cases in Japan have been inoculated with small quantities (2 milligrams to each case) of killed agar culture of the pest bacillus.

The protective which has obviously most widely been used in man is that recommended by Haffkine, and this I believe chiefly to be due to the fact that Haffkine has resided in India and has had abundant opportunity to employ his method. It is not my purpose here to enter into a discussion of the available statistics of the results of the human inoculations performed in India. They are to be found in the publications of the Indian Plague Commission, in other Indian government reports, and in Haffkine's very recent article on the subject.[23] From their study one becomes convinced that it is very difficult to decide just what the value of the inoculations performed by this method has been. However,

[22] In the eighteenth century some desultory attempts were made to secure immunity in man by exposing the individual to direct infection with plague pus. These methods were soon abandoned owing to the disastrous results following their employment. I have previously considered them elsewhere. *This Journal* (1906) 1, 181.

[23] *Bull. de l'inst. Pasteur* (1906), 4, 825.

it seems unquestionable that large and repeated doses of Haffkine's prophylactic frequently protect individuals against plague infection, although it is well known that many of the inoculated persons, including even a number of those who received more than one injection of the prophylactic, have contracted the disease. Thus, the Indian Plague Commission found that some individuals who during two years had received four injections of the prophylactic, fell victims to pest; and, on the other hand, as many as 8 per cent of the inoculated in one district, Bulsar, contracted the disease. In 1902 it was the intention of the Indian government to inoculate the inhabitants of a province containing about six millions of people with this prophylactic. However, the work was discontinued owing to 18 deaths from tetanus which occurred among the inoculated shorty after the project was undertaken.

It is important in discussing the value of Haffkine's inoculations to consider the work of Kolle and Otto [24] on the immunization of guinea pigs with Haffkine's prophylactic. These authors conclusively showed that guinea pigs could not, except in rare instances, be immunized against pest infection by the use of this prophylactic and their experiments led them to emphasize the fact that if large and repeated doses of the killed pest organism failed to immunize such small animals as guinea pigs, it seemed unreasonable to expect very favorable results in man from such a method, particularly since the amount of the bacteria inoculated in human beings is so much smaller in proportion to the body weight.

However, the subject with which I was at first most concerned was work which would lead to a decision as to the *most effective* method of inoculation against pest and this it seemed could best be carried out by experimental studies on animals.

It therefore was from this standpoint that I decided first to investigate the subject.

[24] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), 45,

III. CULTURES EMPLOYED IN THE EXPERIMENTAL WORK.

The cultures of *Bacillus pestis* employed in the experimental work considered in this article consist of one virulent and three avirulent strains of the organism, which will be designated in the descriptions as "Pest Virulent," "Pest Avirulent," "Maassen Alt," and "Avirulent Manila."

"Pest Virulent."—The strain "Pest Virulent" was obtained from a human case of plague which succumbed in October, 1905. It was isolated from the spleen, at autopsy, by plate cultures and was then grown upon an agar slant for one generation. From this culture it was inoculated into a guinea pig. Upon the death of this animal, a portion of its spleen was rubbed over a freshly shaved area on the abdomen of the second guinea pig and this process of inoculation from the spleen of one animal to the abdomen of a fresh one has successively been repeated from animal to animal without an interruption for more than a year and for over two hundred passages of the strain. (See Series of inoculations, p. 296.) Whenever it was desirable to experiment with this strain, cultures were made upon agar from the heart's blood of one of this series of guinea pigs on the day of the death of the animal, and twenty-four hours later the growth, if pure, was transferred to a second agar tube. A second transfer of this culture (never removed longer than three or four days and usually not longer than seventy-two hours from the animal body) was used in my experiments in testing the immunity of the animals inoculated by the different methods, excepting in a few instances in which a first transfer was employed; in these cases it is so stated in the description of the experiments. Hence, the method of testing was uniform, a 48-hour slant agar culture of the first or second transfer invariably being employed. In the preparation of all the prophylactics with which I experimented, in the manufacture of which it was desired to use a virulent organism, a first or second transfer of this culture was also employed. The strain "Pest Virulent" kills guinea pigs, usually in from three to seven days after cutaneous inoculation by massaging the shaved abdomen of the animal with a portion of a plague spleen from another one (see p. 296), and hence it represents a very virulent strain of *Bacillus pestis*. It may be seen from the experiments which will later be recorded in detail that, during the entire time of its passage through guinea pigs, the virulence of this strain for these animals and for rats and monkeys re-

mained unchanged. It is perhaps needless to add that its morphology and cultural characteristics are typical of other unquestionable strains of *Bacillus pestis* and that it is agglutinated by a standard pest serum. A small standard oese was employed throughout the work, of such a caliber that one 48-hour second-generation culture of this strain yielded about 10 oesen of growth.

"Pest Avirulent."—This strain was obtained through the kindness of Prof. W. Kolle, at present Director of the *Institut für Infektionskrankheiten,* in Bern. It represents an attenuated strain of "Maassen Alt," presently to be described. It forms colonies on agar typical of other strains of *Bacillus pestis* and in other ordinary media its growth resembles this organism. Its morphology on agar, while not perfectly typical of *Bacillus pestis,* is suggestive of this organism, since a number of bipolar staining bacilli may be distinguished in the cover slips made from the fresh culture.

A 19-hour agar slant culture of this organism was suspended in bouillon and inoculated beneath the skin of the shaved abdomen of a monkey. Six hours later an incision was made near the point of inoculation and cover slip preparations and cultures secured from the drops of blood which escaped from the incision. No bacilli were found in the cover slips, but the cultures developed numerous colonies presenting the typical appearance of those of *Bacillus pestis.* Microscopical preparations from these colonies revealed plump bacilli, a few involution forms, and others with typical bipolar staining. Some of the organisms occurred singly, others in pairs or rarely in chains of three or four. Some of them appeared encapsulated. In other instances also in which very large amounts of this strain were inoculated intraperitoneally into guinea pigs and in which the animal succumbed to pest intoxication, organisms with the typical morphology of *B. pestis* have also been observed in microscopical preparations made from the abdominal cavity. (See Series 32.) The strain "Pest Avirulent" is agglutinated by two standard pest sera. Its virulence is so reduced that from 1 to 2 whole agar slant cultures do not cause the death of guinea pigs when injected subcutaneously. In small animals under 175 grams in weight, one agar slant injected *intraperitoneally* has occasionally caused death from plague *intoxication.* (See Series 37, animal, number 2043, p. 207.) The unquestionable proof that the organism really represents an attenuated strain of the genuine pest bacillus is furnished by the fact that monkeys and guinea pigs vaccinated with this culture have later been shown to possess high and undoubted pest immunity, by subsequently inoculating them with multiple lethal doses of the strain "Pest Virulent" already described. (See Series 4, 11, 12, 18, etc., pp. 199 to 212.)

Pest "Maassen Alt."—This strain was also obtained by me from Professor Kolle, who originally received it from Dr. Maassen. Both it and

the previous strain have been described by Kolle and Otto,[25] and extensively employed by them in the immunization of various laboratory animals. The cultural peculiarities and morphology of pest "Maassen Alt" are typical of *Bacillus pestis* and microscopical preparations from its cultures show the characteristic bipolar staining of the bacilli. Such forms are particularly noticeable after the bacillus has been inoculated into the subcutaneous tissues of an animal and several hours later reclaimed in cultures. This organism is also agglutinated by two standard pest sera; it likewise represents a much attenuated strain of *Bacillus pestis*, although its virulence is not so far reduced as is that of the strain "Pest Avirulent." Guinea pigs of from 200 to 300 grams weight as a rule recover from subcutaneous injections of such large amounts as from one to two agar slant cultures, yet they sometimes succumb to such inoculations. Evidences of a subacute pest infection are visible in the event of their death. In other experiments, when as much as a whole agar slant culture has been injected intraperitoneally, or more rarely subcutaneously, the animal has died within twenty-four to forty-eight hours, apparently of a toxæmia, the organisms not having multipled to any demonstrable extent, or at least they have certainly not invaded the circulation or the solid organs. This has been demonstrated by the sterility of cultures made from these locations. (See Series 38, animal number 2050, p. 219, and Series 40, animal number 2128, etc., p. 220.) In still other instances in which the guinea pigs have lived six or more days after inoculation and then succumbed and in which the injection has been made subcutaneously, pest bacilli may be demonstrated in cultures from the buboes which have developed but they may not be encountered in the blood of the heart or any of the other organs of the body. As in the case of the strain "Pest Avirulent," undoubted proof that the culture "Pest Maassen" represents an attenuated type of *Bacillus pestis* has been given by the fact that numerous guinea pigs and monkeys have been vaccinated with it and have later shown high and undoubted immunity against inoculations with the strain "Pest Virulent." (See Series 17, 21, etc., pp. 212 to 221.)

Pest "Avirulent Manila."—The culture "Avirulent Manila" was obtained from an autopsy upon a typical case of human bubonic plague occurring in Manila in the autumn of 1903. Its exact virulence at the time of its isolation was not known. It was preserved in the laboratory as a stock laboratory plague culture by Mr. Hare, formerly of this Institute, and was transplanted from time to time on agar. It had not been passed through animals since its isolation up to the time these experiments were begun. In the summer of the year 1905, from 1 to 2

[25] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 513.

oesen of this culture constituted a lethal dose for guinea pigs of 250 to 300 grams weight, when inoculated subcutaneously, the animals usually dying from subacute plague infection. The virulence of this organism has still further been reduced by growing it at a temperature of from 41° to 43° C. in flasks of alcoholic bouillon for three weeks at a time as recommended by Hetsch.[26] (See p. 310, "Virulence of Pest Bacillus.") By this means a considerable reduction in virulence has occurred, since at the present time guinea pigs of 250 to 300 grams weight are usually able to withstand the subcutaneous inoculation of one entire 24-hour agar slant culture of this organism. However, occasionally they succumb to pest infection from a such dose, although the course of the disease in these instances is always prolonged. This organism also shows the typical morphology of *Bacillus pestis* and agglutinates with a standard pest serum; animals vaccinated with it also acquire pest immunity. As this strain was known formerly to have possessed a greater virulence in nature and had been artificially attenuated, experiments were performed with it in monkeys to ascertain whether it would be possible to cause it to regain its original virulence. However, from this standpoint these experiments were unsuccessful. (See p. 301.)

[26] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 442.

IV. ANIMALS EMPLOYED—THEIR SUSCEPTIBILITY TO PEST INFECTION—LETHAL DOSE—TECHNIQUE OF INFECTION.

Rats and monkeys, in addition to guinea pigs and rabbits, mainly have been employed in the experimental work.

Rats.—The rats used in the majority of cases were the ordinary wild ones found in Manila belonging to the species *Mus decumanus,* and in a few instances to the species *M. rattus.* They contract pest infection naturally and have been known to be concerned in the spread of plague in Manila since 1902. During that year the pest bacillus was found in the organs of 0.34 per cent and in the following year in 0.0625 per cent of these rodents collected from various portions of the city of Manila and sent by the Board of Health to the laboratory for examination. The majority of the rats so infected had been found dead or were captured in the houses where cases of human plague had occurred. Since the decline of plague in Manila, only an occasional rat has been found to be infected. Wherry reported from this laboratory one such instance in 1905, and during the present year (1906) the pest bacillus has been isolated but twice from rats collected in Manila and sent to the laboratory for examination. These wild rats could be obtained alive in great abundance and on account of the frequent scarcity and the difficulty of breeding white rats here in this city, the wild species was used in the majority of the plague experiments and they proved very satisfactory animals. The rats were always kept for some time after their capture before being employed for experimental purposes. They were satisfactorily handled during the inoculations by two Filipino assistants, who protected themselves from being bitten by wearing extra heavy leather gloves. Rat tongs or pincers were not employed because of the danger of traumatic injuries to the animals. As was expected, the species *M. decumanus* proved itself to be readily susceptible to plague infection. In the experimental subcutaneous inoculations of these rats a second transfer of a 48-hour culture of "Pest Virulent" was suspended in 5 cubic centimeters of bouillon or saline solution and a syringe needle from a 5 cubic centimeter syringe dipped in this suspension and then thrust beneath the skin near the base of the tail of the rat. After such an inoculation the animal almost invariably succumbed to pest infection, usually in from three and one-half to five days, although life sometimes was prolonged to from the seventh to the fifteenth day after inoculation.

A single animal which evidently possessed unusual resistance or acquired immunity, lived for twenty-two days and finally died of chronic pest.

Chronic forms of pest, such as have been described by Kolle and Martini,[27] in which the inoculated rats lived for months, have not been observed in any of the animals injected with the suspension of the strain "Pest Virulent" in the doses mentioned above. It seems not unlikely that less virulent strains of the organism have usually given rise to the chronic infections in rats. Whenever the death of the rat occurred from the infection, pest bacilli were always found at autopsy in the tissues near the point of inoculation and almost always in the spleen. However, in the spleen the organisms were frequently not so numerous as near the point of inoculation, and in the heart's blood, while they usually were present in abundance, sometimes they were found only in small numbers and occasionally they even were absent.

The Japanese variety of white rats which was used was probably the albino variety of *Mus rattus*. They also almost invariably succumbed to pest infection in a manner similar to the wild rats and they seemed to be equally or somewhat more susceptible to the same amount of the strain "Pest Virulent" than the species of wild rat (*Mus decumanus*).

Whether the Manila wild rat through its progenitors has gradually acquired a slightly greater insusceptibility to pest, a disease to which it has been exposed from time to time, can not be stated. Albrecht and Gohn found gray rats slightly more susceptible than white ones, although the experiments upon which they based their conclusions were not numerous.

Monkeys.—The study of pest infection in monkeys is particularly important, since these animals suffer with forms of the infection analogous to those seen in man and, moreover, are said sometimes to contract the disease naturally. I have never observed a case of spontaneous infection in a monkey in Manila, but I have never systematically sought for such an infection.

In the report of the Indian Plague Commission there is some evidence of the occurrence of plague in monkeys. Most of it is not entirely conclusive, but in some instances the diagnosis was confirmed by bacteriological examination. Albrecht and Gohn [28] have also mentioned spontaneous infection in these animals and Simond [29] and Clemow [30] have reported epidemics among them.

The latter author on three occasions observed that monkeys which were supposed to be of the species *Macacus sinicus*, sickened spontaneously and died. Plague bacilli were isolated from a number of the animals at autopsy.

[27] *Deutsche med. Wchnschr.* (1902), **28**, 3.

[28] Uber die Beulenpest in Bombay: *Denkschr. d. math.-naturw. Klasse d. Kais. Akad.*, Wien (1898–1900), **66**, 726.

[29] *Ann. de l'inst. Pasteur* (1898), **12**, 664.

[30] *Brit. Med. Journ.* (1900), 1141.

Statements in the literature in regard to the lethal dose of pest bacilli for monkeys of different species vary somewhat and are not entirely definite.

Albrecht and Gohn [31] (Report of the Austrian Plague Commission) in their experiments with monkeys used the small, long-tailed, brown Indian species. Inoculations of living cultures in amounts of from 0.5 to 4 oesen were made. With some strains 0.5 oese killed animals after forty-eight hours and with others 2 oesen failed to kill. It is not possible from their experiments to arrive at a general determination of a lethal dose for these animals, since strains of different virulence and in different amounts and with different methods of inoculation were employed and no series of animals by any one method of experimentation is given.

Wyssokowitz and Zabolotny [32] (Russian Plague Commission) in their experiments used three Indian species, described as the *Macacus* with the long tail, the *Macacus* with the short tail and the black ape with a long tail. The two latter were found to be more susceptible to pest infection, dying in from two and one-half to three days, while the former usually succumbed in from four to five days after inoculation. These monkeys proved to be very sensitive to small amounts of plague bacilli. A prick on the palm of the hand or sole of the foot of the animal with a needle which had been moistened with a plague culture, invariably produced death in from three to ten days.

In 1901 Zabolotny,[33] in further experiments, confirmed the fact that in the animals of both the species *Macacus radiatus* and *Semnopithecus entellus* a small amount of pest bacilli gave rise to fatal infection, but that the latter species was more susceptible and succumbed in a shorter time.

The German Plague Commission used two varieties of monkeys in their experiments, *Macacus radiatus* and *Semnopithecus entellus*. The later species was extraordinarily sensitive to pest infection, succumbing in six days to the subcutaneous injection of 0.01 or 0.001 oese of the virulent plague bacillus and dying of larger inoculations after two days. These monkeys also succumbed to pest infection if small amounts of pest bouillon cultures were rubbed into superficial wounds of the skin. The species *Macacus radiatus* was much less susceptible. When the skin was scarified and a fresh agar culture rubbed over the wounded area, or 0.01 of an oese of an agar culture injected subcutaneously, the animals acquired a moderate pest infection, with fever, glandular swellings, etc., but usually survived. However, 1 oese of a two-day agar culture suspended in 1 cubic centimeter of bouillon and injected subcutaneously, always caused the death of this species in three or four days, and $\frac{1}{2}$ or $\frac{1}{4}$ of an oese always brought about the same result, although in a slightly longer time.

In the report of the Indian Plague Commission mention is merely made of the fact that the gray Indian monkey is more susceptible to plague than the brown.

Only the common Philippine species (*Cynomolgus philippinensis* Geoff.) was employed in my experiments with monkeys. The similarity of pest infection in these monkeys to that observed in human beings is very marked, and in the experimental work with them all forms of infection with plague have been encountered. This species of monkey appears to stand between *Macacus radiatus* Geoff. (*Macacus sinicus* Linn) and

[31] *Loc. cit.*, 713.
[32] *Ann. d. l'inst. Pasteur* (1897), **11**, 663.
[33] *Arch. Sci. Biologiques* (1901), **8**, 390.

Semnopithecus entellus Cuvier in relation to susceptibility to plague infection. However, since all three of these species apparently belong to different genera, it is perhaps not strange that their susceptibility to the infection should vary to a certain extent.

Obviously, in the beginning of this work it was important to determine the lethal dose with a pest culture of known virulence for the species *Cynomolgus philippinensis* with which the experiments were to be performed and this was done as accurately as possible. However, the susceptibility of the individual monkeys of this same species was found to vary considerably. Usually, the animal dies from pest infection in from three to seven days if the growth from a 48-hour agar slant culture of the strain "Pest Virulent" is suspended in 5 cubic centimeters of bouillon, and a 5 or 10 cubic centimeter syringe needle dipped in this suspension and then thrust beneath the skin near the root of the tail. Also, if the skin is shaved over a small area and slightly scarified and then a suspension in bouillon of the same culture is rubbed over this area, the animal usually succumbs. On the other hand, some of these monkeys will survive the inoculation of similar and of even much larger amounts of the virulent pest organism, certain of them remaining alive after the injection of even $\frac{1}{4}$ and $\frac{1}{3}$ oese of "Pest Virulent". However, it has been found that $\frac{1}{2}$ oese of "Pest Virulent" suspended in 0.25 cubic centimeter of bouillon always constitutes a fatal dose for monkeys averaging about 2,000 to 3,000 grams in weight, and therefore, although usually this quantity of plague culture really represents many times the multiple lethal dose for the majority of these animals, it has been employed in testing the immunity of all the monkeys used in my experiments and weighing under 3,000 grams, excepting in the first series of experiments where the lethal dose had not been determined accurately and where it is then noted in the tables. For monkeys over this weight $\frac{2}{3}$ oese of "Pest Virulent" suspended in 0.33 cubic centimeter of bouillon has been employed. In order that the size of dose used in testing all the animals might be as uniform as possible one 48-hour agar slant culture of the strain "Pest Virulent" was suspended in 5 cubic centimeters of bouillon or saline solution; 0.25 cubic centimeter of the suspension then contains $\frac{1}{2}$ oese and 0.33 cubic centimeter $\frac{2}{3}$ oese of this organism.

The variations in susceptibility between different individuals of this species of monkey are evidently of considerable importance in the study of their immunization and, as will be seen from the experiments which will be related further on, this individual variation probably accounts largely for the different results in immunization which have been obtained in animals of the same weight and inoculated with the same dose and by the same method.

If, for example, each animal of a series is inoculated with a fixed amount of plague vaccine and, later, the immunity of each is tested by

the inoculation of equal amounts of the virulent pest bacillus, if the susceptibility of the different animals varies, then different results in mortality must occur both for the reason that different degrees of immunity will arise in the different animals from the primary inoculation of the same dose of the vaccine and for the reason that in testing the immunity, the fixed amount of the virulent organism employed will represent in the more susceptible animals a greater multiple of the lethal dose than it does in the less susceptible ones. This variation in susceptibility to the action of the plague bacillus has been found to be much more marked in the monkeys I have used than it is with other laboratory animals, and it seems not unlikely that the conditions relating to susceptibility in these animals approach nearer to those which exist in man than they do to those which are present in such animals as guinea pigs, rats, mice, etc. During the past year we have had numerous opportunities to observe the differences in immunity obtained in different human beings by the inoculation of the same sized dose of our cholera prophylactic, by studying the blood serum from individuals vaccinated against Asiatic cholera. These variations in some instances have been very decided and have been much greater than those which have been observed in series of guinea pigs or of rabbits, all inoculated with an equal dose. Hence, the limit of value of a method for the immunization of man against plague can probably better be studied in monkeys than in any of the other lower animals. It is particularly for this reason and because of the fact that monkeys suffer with forms of plague analogous to those observed in human beings, that they have been extensively used in my experiments in testing the final value of methods of pest inoculation which have proved effective in the ordinary laboratory animals. Moreover, another reason for the extensive use of monkeys has been that it might be argued that the value of a method of immunization against plague in man should not be judged by its action in experiments upon such animals as guinea pigs and mice alone, an argument which has already been advanced.

Guinea pigs and rabbits.—Two methods of infection were employed in guinea pigs, for the purpose of testing their immunity following the prophylactic injections in the various experiments. The first consisted of the suspension of a 48-hour agar culture of the strain "Pest Virulent" in 5 cubic centimeters of bouillon. Five large oesen of this suspension were then rubbed over a shaved area of the abdomen of the animal and the skin scarified with a scalpel. The other method of infection less commonly employed consisted of massaging over a shaved area of the abdomen of the guinea pig a portion of the spleen of a second one just dead from acute pest infection with the strain "Pest Virulent." By either of these methods the guinea pig, unless previously immunized, invariably succumbed to acute infection. Rabbits were only employed in

certain of the agglutinative experiments and in the preparation of aggressin exudates, under which subjects the technique of the inoculations is described.

In testing the immunity of all the animals care was taken to introduce the infection upon the opposite side of the body to that upon which the vaccination or prophylactic injection had first been made. This precaution was taken in order to avoid any chance of obtaining results which might have been influenced by the presence of a local immunity which had developed in the animal, particularly in the tissues about the point where the first inoculation had been made. Wassermann and Citron[34] have recently called special attention to the local development of immune bodies.

[34] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1905), **53**, 331.

V. IMMUNIZATION OF ANIMALS.

1. WITH KILLED PEST BACILLI.

Yersin, Calmette and Borrel[35] in 1895 first called attention to the fact that rabbits, inoculated subcutaneously on three or four occasions with gelatine cultures of the pest bacillus killed by heating for one hour at 58° C., were rendered immune to subsequent subcutaneous inoculations of virulent plague bacilli. Guinea pigs were much more difficult to immunize, and they rarely succeeded in thoroughly protecting one of these animals by such a method.

Wyssokowitz and Zabolotny[36] mention that monkeys can be immunized against pest by the inoculation of killed cultures of the organism, although they give no details showing the percentage of animals that were protected by the use of this method.

The German Plague Commission[37] found that all the monkeys of the species *Macacus radiatus* which received subcutaneously one carefully killed, two-day culture of the virulent pest organism were able later to resist almost without reaction the subcutaneous injection of one full oese of the living culture. However, with this same amount they were not able to immunize gray monkeys of the species *Semnopithecus entellus* against plague infection, and the Commission did not have time to pursue the question further with this species of animal. However, they demonstrated in a series of rat inoculations that a large percentage of the animals could be immunized with killed cultures of the pest organism against subsequent subcutaneous infection, if the dose was sufficiently large (2 killed agar cultures). One agar culture did not suffice to immunize the animal. The rats often succumbed from the effect of the large dose of the primary inoculation.

Albrecht and Gohn[37] state that they were able to immunize guinea pigs by repeated doses of killed agar cultures of the pest bacillus, although the immunity obtained was not great. In their experiments with these animals, described in their report, the guinea pigs all finally died of pest infection.

Tavel, Krumbein and Glucksmann[38] were able to obtain immunity in some instances in the course of a small number of experiments with rats, by the inoculation of large amounts of the killed cultures, but they were unable in many experiments to immunize a single guinea pig even by using repeated inoculations of the killed cultures.

Beinarowitch[39] concluded that the injection of killed cultures of the pest bacillus conferred an immunity upon rodents, but that this was very slight, unless the inoculation was repeated several times.

[35] *Ann. d. l'inst. Pasteur* (1895), **9**, 589.
[36] *Ibid* (1897), **11**, 667.
[37] *Loc. cit.*
[38] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1902), **40**, 239.
[39] *Arch. d. Sci. Biologiques* (1903), **9**, 343.

The most important and most convincing experiments in regard to the value of immunization with killed cultures of the plague bacillus in rats and guinea pigs have been made by Kolle and Otto.[40] In their experiments the loss *during the process of immunization* of the rats with Haffkine's prophylactic was 38.5 per cent and with the killed agar cultures 33.3 per cent.

Later, on testing the immunity of the animals remaining alive, it was found that only 21.9 per cent of those which had been inoculated with the killed agar cultures, and but 22.2 per cent of those inoculated with Haffkine's prophylactic, gave evidence of an acquired immunity by surviving the test. Attempts were made to immunize 26 guinea pigs with killed agar cultures, amounts as large as from ½ to 1 entire agar culture being injected subcutaneously. During the process of immunization four of the animals died. Of the remaining twenty-two, only two (7.7 per cent of the whole) appeared to be immune on subsequent testing. Hardly more favorable results were obtained in the experiments in which killed bouillon cultures were employed. Twenty animals were inoculated with Haffkine's prophylactic. Two of these died from the effects of the immunization and of the remaining eighteen, only two (10 per cent) remained alive after reinoculation with the virulent organism. Other experiments on guinea pigs were also performed in which repeated inoculations were made with killed cultures of the plague organism. The animals were first injected with 1, then with 1½ and finally with 2 killed agar cultures or with 1, 1½ and 3 cubic centimeters of Haffkine's prophylactic. In the process of immunizing twenty guinea pigs by these methods, six of the animals died from the effects of such large doses of the killed bacteria. The immunity of the remaining fourteen was tested six weeks after the last injection with the living virulent plague bacillus when only one animal remained alive and proved to be immune.

In my own experiments the value of inoculation with killed cultures of the pest bacillus was tested on monkeys and guinea pigs.

ANIMAL EXPERIMENTS.

A. With monkeys.—It seemed desirable to experiment further with killed cultures of the pest bacillus for two reasons, first, to compare the immunizing value of the dead organism with that of other methods of inoculation, such as those of vaccination, natural and artificial aggressin injections etc., and second to see whether sufficiently good results could be obtained by experiments on Manila monkeys to warrant advocating the use of this method in man.

In Series 5, page 189 are given the results of the experiments on eight monkeys inoculated with killed bouillon cultures (Haffkine's method) in varying amounts. On testing the immunity of these animals by thrusting a syringe needle infected with virulent pest bacilli beneath the skin, eleven days after the primary inoculation, only three were found to possess sufficient immunity to survive the infection. Of those which died, one had previously received 30 cubic centimeters and another 20 cubic centimeters of the prophylactic. From what has been said under the discussion of the susceptibility of this species of monkey to pest infection, it will be seen that the method of testing the immunity of the

[40] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 512; (1904), **48**, 399.

animals employed in this series was not severe, since in some instances, monkeys which had not been protected at all, survived such a method of infection (for an example of this fact see Series 16, p. 188, animals numbered 1357 and 1358). However, it is true that the two control animals inoculated in Series 5 died of pest infection. In Series 49 (p. 197), nine other monkeys were inoculated by the same method, from 10 to 15 cubic centimeters being injected in each instance. One month after the inoculation, the immunity of the animals was tested and only two were found to resist the infection (numbers 2699 and 2703).

Because of the superiority of the killed agar cultures over the killed bouillon ones, the reasons for which have already been pointed out, only the former were employed in the remainder of the experiments of this nature.

Three monkeys in Series 9 (p. 190) were inoculated, each with two 48-hour killed agar cultures of the strain "Pest Virulent." Ten days later, upon the injection of 2 oesen of the living virulent organism, all the animals succumbed to the resulting infection. The amount used in testing the immunity of these animals obviously was excessively large, as was shown by later experiments; therefore, the series does not represent a fair test of the value of this method of immunization.

The experiments recorded in Series 23 (p. 191) comprise 18 monkeys besides the control animals. The pest organisms in the suspension were not all killed by heating before inoculation, and hence no accurate conclusions can be drawn from these experiments in regard to the immunity produced by the killed organism alone. The size of the dose used in testing the immunity of the animals was also not sufficiently large. (See remark under Series 23.)

In Series 25 (p. 192), twenty monkeys were inoculated with from one to two 48-hour agar cultures of the virulent, killed organism. Three of the animals succumbed from the effects of this inoculation. The immunity of the remaining seventeen was tested eighteen days after the primary inoculation, when but four (23 per cent) resisted the infection and remained alive.

In Series 48 (p. 195), fifteen monkeys were inoculated with one 48-hour agar slant of the killed virulent strain. One of these animals succumbed, its death being apparently caused by this inoculation. The immunity of the remaining fourteen was tested one month after the first injection when but four, or 28 per cent, remained alive.

B. With guinea pigs.—In Series 50 (p. 197), fifteen guinea pigs were inoculated, each with one 48-hour agar culture of the killed virulent organism. Upon testing the immunity of these animals one month later, but four, or 26 per cent, resisted the infection and remained alive.

No further attempts to immunize larger series of guinea pigs were made with killed cultures since, reasoning from the experiments of other observers as well as from my own, it appears that it is possible to immunize

only a small percentage of these animals by such a process. The guinea pigs comprising Series 50 were inoculated with the killed agar cultures, chiefly for the purpose of comparing this method with the other methods of inoculation which were being tested at the same time.

In all, fifteen guinea pigs and seventy-three monkeys were inoculated with killed cultures of the pest bacillus; 26 per cent of the guinea pigs and 32 per cent of the monkeys subsequently proved to be immune. However, if Series 23 is excluded as it should be, since probably both killed and living organisms were used in the immunization of the animals, the percentage of immunity in the remaining monkeys is but 25 per cent.

2. INOCULATIONS IN ANIMALS WITH LIVING ATTENUATED CULTURES (VACCINATION).

I have recently [41] reviewed the literature regarding the early experiments made to attenuate virulent strains of the pest bacillus and of the inoculations performed in animals with other avirulent strains of this organism, and it is not my intention here to enter into any lengthy discussion of this subject but merely to mention these experiments.

The German Plague Commission made some attempts at attenuating strains of the pest bacillus for use in immunization, by exposing the living cultures both to a temperature of 51° C. for varying periods of time and also to the action of carbolic acid. These experiments resulted unsuccessfully, the organisms retaining their full virulence. Albrecht and Gohn [42] and Yersin and Carré [43] also performed experiments on guinea pigs, rats and monkeys with somewhat attenuated pest cultures. No extensive or convincing experiments in regard to the value of the living, attenuated cultures in the immunization of animals against plague had apparently been undertaken until Kolle and Otto investigated this subject. These authors, in numerous and careful experiments on rats and guinea pigs, showed that cultures of the pest bacillus so attenuated that they were no longer dangerous to these animals even in large amounts (2 agar cultures) were capable upon injection of giving rise in them to a much higher immunity than was produced by the killed cultures of the organism. Thus, in one series of fifty-nine guinea pigs which were immunized by a single inoculation of an attenuated living culture, thirteen died and two were killed for control purposes. On testing the immunity of the remaining forty-four from three to eight months after their vaccination, twenty-eight, or 63.6 per cent, remained alive.

In another series of thirty-four guinea pigs immunized with an attenuated pest culture (Maassen V), of which one died during the process of immunization, twenty-one were reinoculated with the virulent organism from one to four months after their vaccination, and of this number, sixteen (76 per cent) remained alive and five died. Nine other guinea pigs were inoculated with this agar culture and at the same time with plague immune serum. All proved to be immune upon reinoculation with the virulent pest bacillus.

[41] *This Journal* (1906), 1, 182.

[42] *Loc. cit.*

[43] Congress International de Méd. Section de Méd. et Chirug. Militaires. Sous section Coloniale Paris (1904), 54.

ANIMAL EXPERIMENTS.

As with the killed pest organism, monkeys and guinea pigs were employed in my experiments on animals with attenuated cultures of the pest bacillus.

Experiments on the immunization of monkeys with the strain "Pest Avirulent."—Six monkeys in Series 4 (p. 199) were inoculated subcutaneously, each with one 24-hour agar culture of the strain "Pest Avirulent." The immunity of the animals was tested ten days later, four being inoculated by thrusting beneath the skin a needle infected with virulent pest bacilli and two (numbers 1232 and 1233) by lightly scarifying the abdomen with a scalpel infected with this same organism. Since two control animals (numbers 1286 and 1287) also inoculated at this time in the latter way did not die, the monkeys numbers 1232 and 1233 can not necessarily be considered immune. Three of the four vaccinated animals wounded with the infected needle survived, while but one of four controls inoculated in the same manner recovered. The conditions encountered in this series are somewhat analogous to those met with in Series 5 (p. 189), where the killed organisms were employed.

Five monkeys in Series 11 (p. 200) were vaccinated, each with one 24-hour agar culture of "Pest Avirulent" and eleven days later their immunity was tested by the inoculation of 2 oesen of "Pest Virulent." Four of the animals died and one survived. The course of the disease in three of those which died was prolonged to twice the length of that in the control animals. In two (numbers 1277 and 1280) the blood serum had evidently acquired considerable anti-infectious power against the pest bacillus; this was presumed to be the case from the distribution of the bacteria at autopsy and from the fact that in one instance the heart's blood was sterile and in the other but six colonies of this organism developed in cultures from the heart's blood. Hence, these animals probably died rather from pest toxæmia than of septicæmia. Their blood may have acquired considerable anti-infectious power with but little antitoxic action. Obviously, the dose (2 oesen) employed in testing the immunity was exceedingly large. This series of experiments may be compared with Series 9 (p. 190), in which the animals immunized with dead bacilli all succumbed upon reinoculation with 2 oesen of the virulent strain.

In Series 12 (p. 201), nine monkeys were inoculated subcutaneously with from one to two 24-hour agar cultures of the strain "Pest Avirulent." One of the animals (number 1299) died twelve hours after the vaccination, of a streptococcus and staphylococcus infection which had existed prior to the vaccination. The immunity of the remaining eight was tested ten days after the vaccination, either by the inoculation of $\frac{1}{2}$ or of 1 oese of the strain "Pest Virulent." Four of the animals remained alive and well, and four died. In those which died, the course of the infection was greatly prolonged beyond that in the control monkeys and the pest

bacilli had not as a rule invaded the circulation and other organs to the same extent as they had in the control animals.

Ten monkeys in Series 18 (p. 202) were vaccinated, each with two agar cultures of the strain "Pest Avirulent." Seventeen days later, upon testing the immunity of the animals, five died from the infection and five survived. Animal number 1379 evidently died of plague toxæmia and not of septicæmia.

In Series 51 (p. 203), fifteen monkeys were vaccinated subcutaneously with one or two 48-hour cultures of the strain "Pest Avirulent." The immunity of the animals was tested one month after the vaccination by the subcutaneous inoculation of two-thirds of an oese of the strain "Pest Virulent." Eight of the animals remained alive and seven died.

In all, forty-four monkeys were vaccinated with the strain "Pest Avirulent" and reinoculated with the strain "Pest Virulent;" 52 per cent of these animals proved to be immune.

Experiments with the strain "Pest Avirulent" in guinea pigs.—Seventy-one guinea pigs in Series 32, 37, 39, 41, and 46 p. 204 were inoculated, either intraperitoneally or subcutaneously, with from 1 to 2 agar cultures of the strain "Pest Avirulent." Such large doses as two 48-hour agar cultures, when injected intraperitoneally into the smaller guinea pigs (150 to 175 grams in weight), frequently gave rise to the death of the animals from toxæmia with, however, no evidence that a general invasion by the organisms had taken place. In only one instance (guinea pig number 1985, p. 205) two colonies of "Pest Avirulent" developed in cultures made from the heart at autopsy. Even one agar culture injected *intraperitoneally* caused the death of one small animal from pest intoxication, the animal dying in less than twenty-four hours after the vaccination. However, when the cultures were injected *subcutaneously,* even in small guinea pigs, death never occurred from the effect of the vaccination; this was demonstrated by the inoculation of fifty-one animals. (See Series 39, 41, and 46, pp. 208 to 212.) Five of the entire number of seventy-one guinea pigs vaccinated with this strain died from the intraperitoneal vaccination with 2 agar cultures, one from the intraperitoneal vaccination with 1 agar culture and one from an unknown cause. The remaining sixty-four were tested one and two months after the vaccination, whereupon forty-six (72 per cent) proved to be immune and twenty-five (38 per cent) died. The test of the immunity of the animals was severe and the skin was well scarified in each instance, two parallel incisions being made through the dermis with a scalpel and the suspension of the virulent organism rubbed into the incisions. Every one of 115 control animals, which were inoculated in exactly the same manner and at the same time as the vaccinated animals, died of pest infection. Guinea pig number 2087 was pregnant at the time of its vaccination and gave birth to two healthy young, seven days after its reinoculation.

Experiments in the immunization of monkeys with the strain "Maassen Alt."—Four monkeys in Series 17 (p. 212) were vaccinated, three with 2 agar cultures and one with 1 culture of the strain "Maassen Alt." Twenty days afterwards their immunity was tested by the inoculation of ½ oese of the strain "Pest Virulent," when all which had been vaccinated with the two cultures proved to be thoroughly immune, while the animal which had received only one culture died of pest. Every one of ten control (nonvaccinated) monkeys inoculated in the same manner died of the infection.

Twelve monkeys in Series 21 (p. 213) were vaccinated, each either with 1 or 2 cultures of this same strain, "Maassen Alt." On testing their immunity two weeks later by the inoculation of ½ oese of the strain "Pest Virulent" it was found that of the eight vaccinated with 2 cultures, two died after a somewhat prolonged infection and of the four vaccinated with 1 culture, two also died, one after a prolonged illness. Therefore, the mortality in this series was 33.3 per cent, 66.6 per cent of the monkeys having been immunized. Each of twelve control animals inoculated at the same time succumbed to pest infection.

Eighteen monkeys in Series 24 (p. 215) were inoculated with agar cultures of this organism ("Maassen Alt"), one with ½ a culture, and the remainder with 1 or 2 cultures. Four of these animals died after the vaccination. It seems clear that the animal numbered 1552 died from the effects of the vaccination and in the remaining three monkeys, death probably occurred both from the same cause and from that of infection with pyogenic cocci. Two weeks later on testing the immunity of the nine which were inoculated with 2 agar cultures and which survived the vaccination, all but one (number 1545) were found to resist the infection. This one died of pest. On testing the immunity of the four animals which had been inoculated with 1 culture and of the one which had been given ½ culture, all were found to be immune. Hence the entire mortality in this series was 28 per cent. However, as a number of the control animals inoculated at this time and in the same manner did not die, the remaining 72 per cent of the animals of this series can not be regarded as being of necessity highly immunized.

Fifteen other monkeys in Series 52 (p. 217) were inoculated subcutaneously each with 1 agar culture of the strain "Maassen Alt." One of the animals died thirteen days after vaccination. The cause of death could not be discovered at autopsy. One month after the vaccination, on testing the immunity of the remaining fourteen by the subcutaneous inoculation of ⅔ oese of the strain "Pest Virulent," seven died and seven survived the inoculation.

In all, forty-nine monkeys were vaccinated with the strain "Maassen Alt;" of these, four probably succumbed from the effects of the vaccination and one from an unknown cause. On testing the immunity of the

remaining forty-four, thirteen died and thirty-one (70 per cent) were found to be immune.

Experiments in the immunization of guinea pigs with the strain "Maassen Alt."—Forty-seven guinea pigs in Series 33, 38, 40, and 47 (pp. 218 to 221) were inoculated either intraperitoneally or subcutaneously with 1 agar culture of the strain "Maassent Alt." In the experiments in which the inoculation was made intraperitoneally, a number of the animals died of pest intoxication, namely, three in Series 33 and one in Series 38. In three of these instances the cultures made at autopsy from the heart's blood of the animal remained sterile; in the fourth, no cultures were taken from the heart. Two of the animals died two days after the vaccination and, although in each instance cultures from the heart were sterile, in both cases cultures prepared from the abdominal cavity developed a rich growth of the strain "Maassen Alt." The guinea pigs also occasionally died from the subcutaneous inoculation of only 1 agar culture of the strain "Maassen Alt;" this was true of one in Series 40 and one in Series 47. In both instances the animals succumbed within twenty-four hours after the vaccination, evidently of plague toxæmia. Therefore, the strain "Maassen Alt" is distinctly more virulent and more toxic than "Pest Avirulent." To sum up, six in all of the forty-seven guinea pigs died from the effect of the vaccination. The immunity of the remaining forty-one was tested from one to two months after the vaccination, when only five (12 per cent) died and thirty-six (88 per cent) were shown to have been thoroughly immunized. One hundred and fifteen unvaccinated control guinea pigs were tested in exactly the same manner and at the same time as the vaccinated ones. All of the control animals died of pest infection.

3. IMMUNIZATION WITH FILTERED CULTURES AND EXTRACTS (FREE RECEPTORS) OF THE ORGANISM.

The German Plague Commission (Gaffky, Pfeiffer, Sticker, Dieudonné) reports two experiments in the immunization of *Macacus* monkeys with filtered bouillon cultures of the plague organism.

A ten day's bouillon culture of the virulent bacillus was filtered through a Berkefeld filter, one portion of the filtrate was heated to 60° C. and another mixed with 0.5 per cent carbolic acid and put aside for twenty-four hours. One of the monkeys was inoculated with 5 cubic centimeters of the first portion and the other with 5 cubic centimeters of the second. On subsequently testing the immunity of these animals with 1 oese of the virulent, living pest bacillus, both succumbed to the infection.

The Austrian Plague Commission (Albrecht and Gohn) found that a moderate degree of immunity could be obtained in rats with filtered bouillon cultures of the plague bacillus. The immunity which resulted from the use of the old bouillon cultures was higher than that which came from the young ones, but in neither instance did it equal that which was obtained with killed cultures of the organism.

Kossel and Overbeck[44] also stated that rats could sometimes be immunized with the filtrates of bouillon cultures which were killed by heating at from 56° to 60° C., although no details of the experiments are given. Markl[45] believed that a soluble pest toxin as well as some metabolic product was formed in the filtrate of young bouillon cultures and of those grown at a low temperature, with which antitoxic immunity could be obtained and an antitoxic serum produced in horses. The animals with which he experimented certainly acquired a tolerance against the injection of the filtered cultures, but the immunity against pest *infection* was not tested in any of them; moreover, Kolle[46] was unable to produce a serum of any value with such cultures, although from repeated inoculations of filtrates of bouillon cultures from eight to ten weeks old he was able to obtain a serum of very low agglutinative and protective power. It would appear from these experiments, that the plague bacillus does not readily undergo autolysis and liberate free antigenetic receptors even in old bouillon cultures.

Besredka[47] found that a separation of the pest endotoxin from the bacilli occurred when dried pest bacilli, physiologic salt solution and normal serum of the horse were mixed and allowed to stand over night at the temperature of the ice box.

Upon centrifuging this mixture and separating the bacteria, the clear fluid was found to contain most of the pest toxin. The precipitated bacteria were found to have lost their toxic action to a great extent, but not their immunizing power. Nine mice were each inoculated with 0.002 milligram of these bacilli "atoxique." Five of the animals survived the inoculation and the course of the infection was prolonged in the remaining four.

Owing to the success I had met with in immunizing both man and animals with the free receptors obtained from the cholera spirillum by autolysis, similar experiments were undertaken upon animals with the plague bacillus.

In the first experiments, 48-hour agar slant cultures of the virulent strain were suspended in a small quantity of distilled water, heated during one to two hours at 60° C., and after being placed for several days in the incubator at 37° C., were filtered through a Berkefeld candle.

Immunization of monkeys with the free receptors of the plague bacillus.—Experiments with such filtrates are recorded in Series 7 and 26 pp. 221 to 223. Nine monkeys were inoculated subcutaneously with various amounts of the free receptors. Only two were found to be immune and to survive the infection when their immunity was tested from ten to fourteen days after the first inoculation. The results of these experiments were so unfavorable that attempts to obtain the free receptors in a somewhat different manner were instituted.

The further experiments of this nature with the free receptors of the organism of pest will be described in the next section of this paper devoted to the subject of immunization with plague aggressin, since, owing to the work of Bail and his associates, the term "aggressin" has now become well established in medical literature.

[44] *Hyg. Rund.* (1901), **11**, 103.
[45] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1901), **37**, 401.
[46] Festschrift f. Robert Koch (1903), 352.
[47] *Ann. d. l'inst. Pasteur* (1905), **19**, 479.

4. IMMUNIZATION WITH PLAGUE AGGRESSIN.

Hueppe and Kikuchi were the first to perform experiments with natural plague aggressin in animals and to announce that a certain and not dangerous method had been discovered for immunization against pest. This work has already been referred to in the introduction to this article. Their experiments were few in number but the authors mention that it was their intention merely to call attention to the priority of the use of this method of immunization of animals against plague. The guinea pigs which were immunized had received several inoculations of the aggressin exudates.

(*a*) IMMUNIZATION WITH ARTIFICIAL AGGRESSIN.

Further attempts at immunization with the free receptors of the plague bacillus (artificial plague aggressin) were being performed in this laboratory at the time of the appearance of Hueppe and Kikuchi's work, but no very favorable results could be obtained. After the publication of their article the subject was investigated anew, although I had already made a preliminary report of the value of immunization with *artificial* plague aggressin on March 3, 1906.[48] Further experiments made in immunization with artificial plague aggressin are given in detail in Series 29, 30, 31, 36, and 42 (pp. 223 to 229), together with the method of preparation of the extract of the organism. In all, twenty-six guinea pigs and thirty-two monkeys were inoculated with the extracts of the strain "Pest Virulent." Only three of the guinea pigs (11 per cent) and but four of the monkeys (12.5 per cent) proved to be immune, when the immunity of the animals was tested from two to seven weeks after the first inoculation. These results were so unfavorable as compared with those in which the living, attenuated cultures of the pest organism were used for inoculation, that this method was not pursued further, but experiments with natural plague aggressin were undertaken on guinea pigs for the purpose also of comparing the immunizing power of this substance with that of the living attenuated organism.

(*b*) IMMUNIZATION IN GUINEA PIGS WITH EXUDATES FROM PLAGUE-INFECTED ANIMALS (NATURAL AGGRESSIN).

The protocols of the animals employed are given in Series 34 and 43 (p. 229), where the further details in the preparation of the aggressin exudates are also described. Fifteen of the guinea pigs of Series 35 (p. 230), were inoculated with the exudates obtained from the animals comprising Series 34, the guinea pigs each receiving, intraperitoneally from 2 to 5 cubic centimeters of the exudates. All survived the inoculation of the aggressin. Their immunity was tested with the virulent organism about two months after the first inoculation, when all but four (26+ per

[48] *This Journal* (1906), 1, 501.

cent) succumbed to pest infection. Twenty-seven control animals were also inoculated at the same time in the same manner; these all died of pest. Twelve animals in Series 44 (p. 232) were inoculated with exudates obtained from the guinea pigs comprising Series 43. The immunity of the animals was tested about one month after the first inoculation; eight (66.6 per cent) died and four (33.3 per cent) were found to be immune.

5. IMMUNIZATION OF ANIMALS BY KLEIN'S METHOD.

Only a few guinea pigs were inoculated with the prophylactic prepared after the method of Klein because it did not seem clear that it possessed any advantages over immunization with the aggressin exudates, while it appeared to cause more local irritation. Thirteen guinea pigs in Series 54 (p. 232) and 56 (p. 233) were each inoculated with a portion of the powder dissolved in 2 cubic centimeters of saline solution and obtained from dying at 46° to 47° C. and then triturating in a mortar the buboes, spleen, liver and lungs of guinea pigs which had died of subacute pest infection. Sloughs of the skin followed the inoculations in five instances. The immunity of the animals was tested with the virulent pest strain about one month after the first inoculation, when all but four (30 per cent) succumbed to pest infection.

Klein does not refer to a marked local reaction following the injection of his prophylactic, and perhaps such a reaction was in his experiments avoided by some details in the manufacture of the prophylactic not emphasized in his preliminary description of its preparation.

VI. SERIES OF ANIMAL INOCULATIONS EMPLOYED IN TESTING THE IMMUNIZING VALUE OF THE DIFFERENT METHODS.

EXPERIMENTS DEMONSTRATING SUSCEPTIBILITY OF MONKEYS TO PLAGUE INFECTION.

SERIES 1.—*With monkeys.*

One 48-hour agar slant culture of "Pest Virulent" was suspended in 5 cubic centimeters of bouillon. A 10 cubic centimeter syringe needle was dipped in such a suspension and the needle then thrust beneath the shaved skin of each animal in the region near the root of the tail, pushed in full length and then withdrawn.

Animal No.	Result.	Autopsy and remarks.
1222	Dead in 3 days	Pest septicæmia. *B. pestis* from heart, liver and spleen and hæmorrhagic inguinal glands.
1223	Dead in 4 days	Pest septicæmia. Innumerable pest bacilli in blood of heart.
2695	ditto	Pest septicæmia. *B. pestis* from heart, spleen and liver.
2696	Dead in 3½ days	Pest septicæmia. *B. pestis* from heart, spleen and liver.

SERIES 2.—*With monkeys.*

One 48-hour agar slant culture of "Pest Virulent" (second transplant from guinea pig number 1220) suspended in 5 cubic centimeters of bouillon. One cubic centimeter of the suspension equal to 2 oesen. Animals both inoculated subcutaneously near root of tail with 1 cubic centimeter.

Animal No.	Amount inoculated.	Result.	Autopsy and remarks.
1224	1 cc. (=2 oesen) subcutaneously.	Dead in 3 days.	Pest septicæmia. Smears from the heart, spleen, hæmorrhagic glands and liver show numerous pest bacilli.
1225	ditto	Dead in 2 days.	Pest septicæmia. Hæmorrhagic area about point of inoculation. Left inguinal glands swollen and hæmorrhagic. Spleen swollen. Innumerable pest bacilli from all organs.

SERIES 3.—*With monkeys and guinea pigs.*

One 48-hour agar slant culture of "Pest Virulent" suspended in 5 cubic centimeters of bouillon. A scalpel was dipped several times in such a suspension and then rubbed over a shaved area on the abdomen, which was also slightly scarified with the knife.

Animal No.	Result.	Autopsy and remarks.
Monkey 1226.	Dead in 5 days.	Found dead in the morning. On microscopical examination of stained-blood films from heart's blood, pest bacilli found present in very small numbers. Cultures from the heart developed colonies of the pest bacillus. A portion of the spleen of this animal was rubbed over the shaved abdomen of monkey No. 1244 (see below).
Monkey 1227.	Dead in 4 days.	Plague septicæmia. Hæmorrhagic glands. Cover slip specimens from heart's blood reveal innumerable pest bacilli.
Monkey 1286.	Alive	
Monkey 1287.	do	

The abdomen of each animal was shaved over a small area and scarified lightly, then a portion of the spleen of another animal which had succumbed to acute pest infection was rubbed over this area.

Animal No.	Abdomen massaged with spleen of—	Result.	Autopsy and remarks.
Monkey 1244.	Monkey No. 1226 (see above).	Alive and well.	
Guinea pig 1170.	Monkey No. 1288 (see Series 5, Oct. 25).	Dead Oct. 30, after 5 days.	Buboes and multiple necrotic foci in spleen and liver. Pure cultures of *Bacillus pestis* from the heart.
Guinea pig 1296.	do	do	Buboes and multiple necrotic foci in spleen and liver. Numerous pest bacilli in smears from spleen.

SERIES 16.—*With monkeys.*

On November 2, two 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1296, second transplant) were suspended in 10 cubic centimeters of bouillon (5 cubic centimeters to each tube), a 10 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin of monkeys numbers 1357 to 1360 inclusive. Monkeys numbers 1361 to 1364 in-

SERIES 16—Continued.

clusive each received subcutaneously 0.5 cubic centimeter of this same suspension (equal to 1 oese "Pest Virulent") and monkeys numbered 1365 to 1368 each received subcutaneously 0.25 cubic centimeters, equal to ½ oese.

Animal No.	How inoculated.	Result.	Remarks and autopsy.
1357	Stuck with 10 cc. needle dipped in suspension "Pest Virulent."	Alive and well	Animal suffered a mild infection and became immune, resisting the reinoculation of 2 whole oesen "Pest Virulent" on Dec. 14.
1358	do	do	Do.
1359	do	Dead Nov. 7, after 5 days.	Innumerable pest bacilli in smears from the spleen.
1360	do	Dead Nov. 9, after 7 days.	Innumerable pest bacilli in smears from the spleen. Pest septicæmia.
1361	1 oese "Pest Virulent" subcutaneously.	Dead Nov. 6, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1362	do	Dead Nov. 5, after 3 days.	Do.
1363	do	do	Do.
1364	do	do	Do.
1365	½ oese "Pest Virulent" subcutaneously.	Dead Nov. 7, after 5 days.	Do.
1366	do	do	Do.
1367	do	do	Do.
1368	do	Dead Nov. 6, after 4 days.	Do.

EXPERIMENTS IN IMMUNIZATION OF ANIMALS WITH KILLED CULTURES OF THE PEST BACILLUS.

SERIES 5.—*Killed bouillon cultures, with monkeys.*

The following monkeys were inoculated subcutaneously near the root of the tail with Haffkine's plague prophylactic purchased by the Bureau of Health from India. The immunity of all the animals was tested eleven days after the first injection, in the following manner: One 24-hour agar slant culture of "Pest Virulent" was suspended in 5 cubic centimeters of saline solution; a 10 cubic centimeter syringe needle was dipped in this suspension and then thrust beneath the shaved skin of the animal on the opposite side of the tail to which the first injection had been made.

SERIES 5—Continued.

Animal No.	Amount of prophylactic inoculated.	Result after infection.	Autopsy and remarks.
1234	5 cc	Dead after 5 days	Pest septicæmia. Innumerable pest bacilli in heart's blood.
1235	5 cc	do	Do.
1236	10 cc	do	Numerous pest bacilli in smears from spleen.
1237	30 cc	Dead after 7 days	Extensive hæmorrhages in the mesentery. Numerous pest bacilli in smears from spleen.
1238	20 cc	Dead after 14 days	Only one or two bacilli found in smears from spleen. Innumerable in smears from large necrotic bubo near region of left buttock (point of inoculation)—chronic pest.
1239	20 cc	Alive	
1240	10 cc	do	
1241	30 cc	do	
1288	Control	Dead after 3½ days	Numerous pest bacilli in smears from the blood and spleen.
1289	Control	Dead after 7 days	Numerous pest bacilli in smears from spleen.

SERIES 9.—*Killed agar cultures "Pest Virulent," with monkeys.*

Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1221, second transplant) suspended in 6 cubic centimeters of saline solution (1 cubic centimeter to each tube), the whole mixed and heated for one and one-half hours at 62° C. A culture taken proved the suspension to be sterile. The following animals were inoculated subcutaneously, each with 2 cubic centimeters (equal to 2 agar slant cultures) on October 18. On October 28 the animals were reinoculated subcutaneously on the other side of the body with 1 cubic centimeter of a suspension of a 48-hour slant culture of living "Pest Virulent" in 5 cubic centimeters of bouillon (1 cubic centimeter equal to 2 oesen).

Animal No.	Immunized October 18.	Infected October 28.	Result.	Autopsy and remarks.
1261	2 agar slant cultures.	2 oesen "Pest Virulent" subcutaneously.	Dead after 4 days.	Pest septicæmia.
1262	do	do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1263	do	do	do	Innumerable pest bacilli in smears from spleen.
		CONTROL.		
1322		2 oesen "Pest Virulent" subcutaneously.	Dead after 3 days.	Innumerable pest bacilli in spleen.

SERIES 23.—*Killed and living agar cultures of "Pest Virulent" with monkeys.*

Twenty-five 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1496, second transplant) were suspended in 25 cubic centimeters of bouillon. The suspension was placed in an incubator registering 60° C. for one and one-half hours. After its removal, cultures were taken, which, two days later, developed colonies of pest bacilli (three colonies to two large loops of the suspension.) Evidently, the temperature of the very concentrated suspension of bacilli had not reached 60° C., although a thermometer placed in a test tube containing bouillon in the incubator registered this temperature during the heating. With this suspension containing killed and also a moderate number of living pest bacilli this series of animals was inoculated as below on December 4, 1906. On December 18, two weeks after the first inoculation, the animals which remained alive were reinoculated in the following manner. Nine 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1516, second transplant) were suspended in 45 cubic centimeters of bouillon, and each animal was reinoculated subcutaneously on the opposite side of the body to that on which the first inoculation was made with 0.2 cubic centimeter of this suspension, that is with a little less than ½ oese of "Pest Virulent."

Animal No.	Inoculated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1518	1 killed agar slant "Pest Virulent."		Dead from first inoculation Dec. 6, after 2 days.	A few pest bacilli in smears from the spleen.
1519	do		do	Very numerous pest bacilli in smears from spleen.
1520	do	Nearly ½ oese "Pest Virulent."	Dead Dec. 20	Culture from the spleen negative. Numerous pest bacilli in smears from the bubo.*
1521	do	do	Alive	
1522	do	do	do	
1523	do	do	do	
1524	do	do	do	
1525	do	do	do	
1526	do	do	do	
1527	do		Dead from first inoculation Dec. 6, after 2 days.	Smears from point of inoculation show no bacilli. Cultures from spleen show numerous pest bacilli.
1528	2 killed agar slants "Pest Virulent."	Nearly ½ oese "Pest Virulent."	Dead Jan. 4, after 17 days.	Numerous pest bacilli in smears from spleen. Large suppurating bubo.
1529	do	do	Alive	
1530	do	do	do	
1531	do	do	do	
1532	do	do	do	
1533	do	do	Dead Dec. 24, after 6 days.	Abscess at point of inoculation containing pest bacilli.
1534	do	do	Alive	
1535	½ killed agar slant "Pest Virulent."	do	Dead Dec. 20, after 2 days.	Very numerous pest bacilli in smears from spleen.*

*It is possible that these animals were suffering with a latent or chronic form of pest at the time of the second reinoculation, although they were apparently healthy.

SERIES 23—Continued.

CONTROLS.				
Animal No.	Inoculated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1594		Nearly ⅛ oese "Pest Virulent."	Alive and well	
1595		do	do	
1596		do	do	
1597		do	Dead Dec. 25, after 7 days.	Pest bubo with innumerable bacilli.
1598		do	do	Do.
1599		do	do	Do.
1600		do	do	Pest bubo with innumerable bacilli. Innumerable bacilli in the spleen.
1601		do	Dead Dec. 26, after 8 days.	Do.
1602		do	Alive and well	Do.
1603		do	Dead Jan. 3	No evidence of pest infection.
1604		do	Dead Dec. 27, after 9 days.	Pest bubo containing numerous pest bacilli. No organisms seen in a smear from spleen.
1605		do	Alive and well	

*It is possible that these animals were suffering with a latent or chronic form of pest at the time of the second reinoculation, although they were apparently healthy.

The majority of the monkeys of this series, including the controls, were very large, weighing 5,000 grams and over, the large animals having been collected and saved for inoculation in the same series. A number of the controls, as will be seen from an examination of the table, did not die, and hence the exact value of the immunization is not shown by the experiments. In the series of inoculations performed after this date, whenever the monkey's weight was over 3,000 grams, ⅔ oese of "Pest Virulent" was employed in testing the immunity, in place of ½ oese. However, some of the very large monkeys were apparently as susceptible to pest infection as certain of the small ones. The series on the other hand shows very distinctly an important fact, namely, that it is not possible to immunize all animals of this species with a single uniform dose, for, although the primary inoculation was so large that it killed some of the animals of this series, in other instances it failed to protect them against the subsequent inoculation of even less than ½ oese of "Pest Virulent," which amount, in even some of the control animals, did not give rise to a fatal infection. The animals that succumbed to the first inoculation also demonstrate the inefficacy of a single heating in attenuating the pest bacillus, the few bacilli remaining alive in the suspension having retained their full virulence.

SERIES 25.—*Killed agar cultures of "Pest Virulent" with monkeys.*

On December 8, twenty-eight 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1514, second transplant) were suspended in 28 cubic centimeters of 0.085 saline solution (1 cubic centimeter to each culture) and the suspension placed at 65° C. for one hour. Cultures from this suspension afterwards proved to be sterile. Monkeys numbered 1558 to 1571 inclusive were

SERIES 25—Continued.

inoculated subcutaneously each with 1 cubic centimeter, monkeys numbered 1572 to 1576 each with 2 cubic centimeters and number 1577 with 0.8 cubic centimeter. Three of the animals apparently succumbed from the effect of this inoculation. On December 26, eighteen days after the first inoculation, the animals were reinoculated in the following manner: Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1592, second transplant) were suspended in 30 cubic centimeters bouillon (5 cubic centimeters to each tube). Each animal was inoculated subcutaneously on the opposite side of the body to that on which the first inoculation was made with 0.33 cubic centimeter, equal to $\frac{2}{3}$ oese "Pest Virulent." Twelve control animals were also inoculated at the same time with the same amount of the suspension.

Animal No.	Inoculated December 8 subcutaneously with—	Reinoculated December 26 with—	Result.	Autopsy and remarks.
1558	1 killed culture "Pest Virulent."	$\frac{2}{3}$ oese "Pest Virulent."	Dead Dec. 28, after 2 days.	Innumerable pest bacilli in smears from spleen.
1559	do	do	Dead Jan. 10, after 15 days.	Chronic pest. Very numerous pest bacilli in smears from spleen.
1560	do	do	Alive	
1561	do	do	Dead Dec. 28, after 2 days.	Fair number of pest bacilli in smears from the spleen. Very numerous about point of inoculation. Tissues œdematous and hæmorrhagic.
1562	do	do	Dead Dec, 28, after $2\frac{1}{2}$ days.	Numerous pest bacilli in smears from the bubo. Culture from spleen negative.
1563	do	do	Dead Jan. 5, after 10 days.	Numerous pest bacilli in smears from spleen. Large bubo.
1564	do	do	Alive and well	
1565	do		Died of first inoculation.	Nothing to suggest pest about point of inoculation or in the spleen. Smears from spleen negative.
1566	do	$\frac{2}{3}$ oese "Pest Virulent."	Dead Jan. 4, after 9 days.	Numerous pest bacilli in smears from spleen. Bubo.
1567	do	do	Dead Dec. 30, after 4 days.	Few organisms in smears from the spleen. Innumerable about point of inoculation. Intense hæmorrhagic reaction.
1568	do	do	Dead Dec. 27, after $1\frac{1}{2}$ days.	Innumerable pest bacilli from point of inoculation.
1569	do	do	Dead Jan. 2, after 7 days.	Few bacilli in smears from spleen. Innumerable from bubo.
1570	do		Dead Dec. 11, from primary inoculation.	Nothing to suggest pest except small hæmorrhages in the lungs. Nothing suggestive at the point of inoculation. Cultures from the spleen negative although large amount is inoculated.
1571	do	$\frac{2}{3}$ oese "Pest Virulent."	Alive and well	

SERIES 25—Continued.

Animal No.	Inoculated December 8 subcutaneously with—	Reinoculated December 26 with—	Result.	Autopsy and remarks.
1572	2 killed cultures "Pest Virulent."	----------	Dead Dec. 24, from primary inoculation.	No evidences of pest. Animal emaciated. Tissues softened, hæmorrhagic and necrotic near point of infection.
1573	do	$\frac{2}{3}$ oese "Pest Virulent."	Dead Jan. 6, after 11 days.	Numerous pest bacilli in smears from spleen. Large suppurating bubo.
1574	do	do	Dead Jan. 7, after 12 days.	Numerous pest bacilli in smears from spleen. Large bubo.
1575	do	do	Dead Dec. 31, after 5 days.	Fair numbers of pest bacilli in smears from the spleen.
1576	do	do	Alive and well.	
1577	$\frac{1}{5}$ killed culture "Pest Virulent."	do	Dead Jan. 6, after 11 days.	Fair number of pest bacilli in smears from the spleen. Large suppurating bubo.
		CONTROLS.		
1613	----------	$\frac{2}{3}$ oese "Pest Virulent."	Dead Dec. 30, after 4 days.	Innumerable pest bacilli in smears from spleen.
1614	----------	do	Dead Oct. 29, after 3 days.	Do.
1615	----------	do	Dead Dec. 30, after 4 days.	Fair numbers of pest bacilli in smears from the spleen.
1616	----------	do	Dead Dec. 30, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1617	----------	do	Dead Dec. 29, after 3 days.	Do.
1618	----------	do	Dead Jan. 2, after 7 days.	Very numerous pest bacilli in smears from spleen. Also many post-mortem bacilli.
1619	----------	do	Dead Dec. 31, after 5 days.	Innumerable pest bacilli in smears from spleen.
1620	----------	do	Dead Dec. 28, after 2 days.	Few pest bacilli in smears from spleen. Innumerable in smears near point of inoculation. No pus formation at this point. The tissues hæmorrhagic.
1621	----------	do	do	Considerable number pest bacilli in smears from spleen. Tissues œdematous and hæmorrhagic near point of inoculation in which pest bacilli are very numerous.
1622	----------	do	Dead Jan. 2, after 7 days.	Few pest bacilli in smears from spleen.
1623	----------	do	Dead Jan. 12, after 17 days.	Numerous pest bacilli in smears from spleen. Large open bubo filled with pus.
1624	----------	do	Dead Jan. 4, after 9 days.	Fair numbers of pest bacilli in smears from the spleen. Bubo.

SERIES 48.—*Killed agar cultures of "Pest Virulent" with monkeys.*

Fifteen 48-hour agar cultures of the strain "Pest Virulent" from guinea pig number 2583, second transplant, were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture), the entire suspension heated for one hour and fifteen minutes at 65° C. Cultures taken from the suspension remained sterile.

On October 27 each monkey of the series was inoculated subcutaneously with 1 cubic centimeter of the suspension (equal to one agar slant culture). On November 28, one month after the inoculations, the immunity of the animals was tested by the subcutaneous injection of 0.33 cubic centimeter of a suspension of the strain "Pest Virulent" (five 48-hour agar cultures from guinea pig number 2865, second transplant in 25 cubic centimeters bouillon) into the opposite side of the body to that on which the vaccination was made. Twenty-five control animals were also inoculated on this date and in the same manner. The immunity of the animals of this series was tested on the same date and in the same manner as was that of the animals comprising Series 49, 51, and 52 (pp. 197, 203, 217).

Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2664	One killed 48-hour culture "Pest Virulent."	⅔ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Few pest bacilli in smears from spleen. Numerous at point of inoculation. Buboes.
2665	do	do	Alive and well.	
2666	do	do	do	
2667	do	do	Dead Dec. 1, after 3 days.	Innumerable pest bacilli in smears from spleen.
2668	do	do	do	Innumerable pest bacilli in smears from spleen.
2669	do	do	Dead Dec. 2, after 4 days.	Few pest bacilli in smears from spleen.
2670	do	Not reinoculated.	Dead Nov. 5, of first inoculation.	Careful post-mortem does not disclose cause of death. No marked local reaction.
2671	do	⅔ oese "Pest Virulent."	Dead Dec. 3, after 5 days.	Buboes and pest bacilli.
2672	do	do	Dead Dec. 1, after 3 days.	Do.
2673	do	do	Dead Dec. 3, after 5 days	Do.
2674	do	do	Dead Dec. 1, after 3 days.	Do.
2675	do	do	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
2676	do	do	Alive and well.	
2677	do	do	do	
2678	do	do	Dead Dec. 1, after 3 days.	Fair number of pest bacilli in smears from spleen.

SERIES 48—Continued.

CONTROLS.				
Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2896		⅛ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
2897		do	Dead Dec. 3, after 5 days.	Do.
2898		do	Dead Dec. 1, after 3 days.	Do.
2899		do	Dead Dec. 2, after 4 days.	Do.
2900		do	do	Do.
2901		do	do	Do.
2902		do	Dead Dec. 1, after 3 days.	Do.
2903		do	do	Do.
2904		do	do	Do.
2905		do	Dead Dec. 2, after 4 days.	Do.
2906		do	Dead Dec. 1, after 3 days.	Do.
2907		do	Dead Dec. 2, after 4 days.	Do.
2908		do	do	Do.
2909		do	Dead Dec. 3, after 5 days.	Do.
2910		do	Dead Dec. 1, after 3 days.	Do.
2911		do	Dead Dec. 3, after 5 days.	Do.
2912		do	do	Do.
2913		do	Dead Dec. 2, after 4 days.	Do.
2914		do	Dead Dec. 1, after 3 days.	Do.
2915		do	Dead Dec. 2, after 4 days.	Do.
2916		do	do	Do.
2917		do	Dead Dec. 1, after 3 days.	Do.
2918		do	Dead Dec. 3, after 5 days.	Do.
2919		do	Dead Dec. 4, after 6 days.	Do.
2920		do	Dead Dec. 1, after 3 days.	Do.

SERIES 49.—*Killed bouillon cultures of "Pest Virulent," with monkeys.*

The monkeys of this series were inoculated on October 27, subcutaneously near the root of the tail with from 10 to 15 cubic centimeters of six weeks' old bouillon cultures of the virulent pest organism killed by heating for one hour at 65° C. On November 28, one month after the primary inoculation, the immunity of the animals was tested by the subcutaneous injection of $\frac{2}{3}$ oese of the strain "Pest Virulent" (from a 48-hour agar culture from guinea pig number 2865, second transplant), suspended in 0.33 cubic centimeter saline solution. The animals were inoculated on the opposite side of the body from that upon which the primary inoculation was made. Twenty-five control animals, numbered 2896 to 2920, were also inoculated on this date and in the same manner. They all died of pest infection. For the details of the autopsies see Series 48. The immunity of the animals of this series was tested on the same date and in the same manner as was that of the animals comprising Series 48, 51 and 52, pp. 195, 203, 217.

Animal No.	Inoculated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2697	15 cc. killed bouillon culture.	$\frac{2}{3}$ oese "Pest Virulent."	Dead Dec. 7, after 9 days.	Bubo containing pest bacilli.
2698	do	do	Dead Dec. 5, after 7 days.	Buboes containing pest bacilli.
2699	do	do	Alive and well.	
2700	do	do	Dead Dec. 5, after 7 days.	Do.
2701	do	do	Dead Dec. 8, after 10 days.	Do.
2702	10 cc. killed bouillon culture.	do	Dead Dec. 3, after 5 days.	Do.
2703	do	do	Alive and well.	
2704	do	do	Dead Dec. 1, after 3 days.	Do.
2705	do	do	Dead Dec. 2, after 4 days.	Do.

SERIES 50.—*Killed agar culture of "Pest Virulent," with guinea pigs.*

Fifteen 48-hour agar cultures of the strain "Pest Virulent" from guinea pig number 2583, second transplant, were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). The entire suspension was heated for one hour and fifteen minutes at 65° C. Cultures taken from the suspension remained sterile. On October 27 each guinea pig of the series was inoculated

SERIES 50—Continued.

subcutaneously with 1 cubic centimeter of the suspension, equal to 1 agar slant culture. On November 26, one month after vaccination, the immunity of the animals in each instance was tested with the strain "Pest Virulent" in exactly the same manner as in Series 46, 47, and 54 (pp. 211, 220, 232). Twenty-five control animals, numbered 2870 to 2894, were inoculated at the same time and in the same manner, all of which died of acute plague infection. For the details of the protocols see Series 46 (p. 211).

Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2679	One 48-hour culture killed "Pest Virulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2680	do	do	do	
2681	do	do	do	
2682	do	do	Dead Dec. 4, after 8 days.	Numerous pest bacilli in smears from spleen.
2683	do	do	Dead Dec. 12, after 16 days.	Large bubo. Typical pest spleen.
2684	do	do	Dead Dec. 5, after 9 days.	Typical pest bubo and spleen.
2685	do	do	Dead Dec. 10, after 14 days.	Do.
2686	do	do	Dead Dec. 13, after 17 days.	Multiple buboes. Infarcts in lung and spleen.
2687	do	do	Dead Dec. 10, after 14 days.	Typical bubo and spleen.
2688	do	do	Dead Dec. 4, after 8 days.	Do.
2689	do	do	Dead Dec. 10, after 14 days.	Do.
2690	do	do	Alive and well.	
2691	do	do	Dead Dec. 8, after 12 days.	Do.
2692	do	do	Dead Dec. 4, after 8 days.	Do.
2693	do	do	Dead Dec. 7, after 11 days.	Do.

EXPERIMENTS IN THE IMMUNIZATION OF ANIMALS WITH LIVING, ATTENUATED CULTURES (VACCINATION).

SERIES 4.—*Inoculations with "Pest Avirulent" in monkeys.*

Six 24-hour agar slant cultures of "Pest Avirulent" were suspended in 6 cubic centimeters of bouillon (1 cubic centimeter to each culture), the whole mixed and 1 cubic centimeter of the mixed suspension injected subcutaneously near the root of the tail of each animal on October 10. Ten days later the immunity of the animals was tested in the following manner: A 48-hour agar culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters of bouillon and animals numbered 1228 to 1231 and controls numbered 1282 to 1285 were reinoculated by dipping a 10 cubic centimeter syringe needle in this suspension and then thrusting the needle beneath the shaven skin near the root of the tail, on the opposite side to that upon which the vaccination had been made. Animals numbered 1232 and 1233 and controls numbered 1286 and 1287 were reinoculated by dipping a scalpel several times in this same suspension and rubbing it over the shaved abdomen and slightly scarifying the skin with the knife.

Animal No.	Vaccination October 10.	Reinoculated October 20.	Result.	Autopsy and remarks.
1228	1 agar culture.	By sticking with infected needle.	Dead after 8 days.	Numerous pest bacilli from the spleen.
1229	do	do	Alive	
1230	do	do	do	
1231	do	do	do	
1232	do	By scarification with infected scalpel.	do	
1233	do	do	do	
		CONTROLS.		
1282		By sticking with infected needle.	Dead after 6 days.	Innumerable pest bacilli in smears from heart's blood and spleen.
1283		do	Dead after 8 days.	Numbers of pest bacilli in smears from the spleen.
1284		do	Dead after 10 days.	A few pest bacilli in smears from the spleen. Very numerous in smears from the swollen hæmorrhagic glands.
1285		do	Alive	
1286		By scarification with infected scalpel.	do	
1287		do	do	

SERIES 11.—*Inoculations with "Pest Avirulent" in monkeys.*

Ten 24-hour agar slant cultures (—1 per cent alkaline to phenol phthaleïn)* of the strain "Pest Avirulent" were suspended each in 1 cubic centimeter of saline solution, the whole 10 cubic centimeters mixed and 1 cubic centimeter, or about 1 agar slant, injected into each animal subcutaneously near the root of the tail. Eleven days later the immunity of the animals was tested in the following manner: Two 48-hour agar cultures of "Pest Virulent" (monkey number 1295, second transplant) were suspended each in 5 cubic centimeters of bouillon, and each animal was reinoculated subcutaneously on the opposite side of the body with 1 cubic centimeter of this suspension, equal to 2 oesen.

Animal No.	Vaccination October 19.	Reinoculated October 30.	Result.	Autopsy and remarks.
1277	1 agar culture.	2 oesen "Pest Virulent."	Dead Nov. 6 (7 days).	Smears from the spleen revealed only a few swollen bacilli. In smears from the heart no organisms were found. In smears from the tissues at the point of inoculation the organisms are numerous. Cultures from the heart's blood (2 cultures) negative; plenty of blood inoculated.†
1278	do	do	Alive and well.	
1279	do	do	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from the spleen.
1280	do	do	Dead Nov. 6 (7 days).	Smears from the spleen show only a few degenerating bacilli. Those from the heart show no organisms. Those from the bubo near point of inoculation show numerous pest bacilli. Cultures from the heart reveal 6 colonies only of pest bacilli.†
1281	do	do	Dead Nov. 7 (8 days).	Very numerous pest bacilli in smears from the spleen.
		CONTROLS.		
1325	None	2 oesen "Pest Virulent."	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from the spleen.
1326	do	do	Dead Nov. 1 (2 days).	Innumerable pest bacilli in smears from the spleen. Culture from the heart=rich growth.
1327	do	do	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from spleen.
1328	do	do	do	Do.
1329	do	do	Dead Nov. 3 (4 days).	Do.

*The growth of the pest bacilli on this agar appeared lighter than it did on + 1 per cent acid agar which was used in all the other experiments.

†The blood of these animals has apparently acquired considerable anti-infectious power, if one may judge from the distribution of the bacilli.

SERIES 12.—*Inoculations with "Pest Avirulent" in monkeys.*

Thirteen 24-hour agar slant cultures of "Pest Avirulent" were suspended each in 1 cubic centimeter of bouillon, the suspensions were mixed and monkeys numbered 1298 to 1301 inclusive were each inoculated subcutaneously with 2 cubic centimeters and monkeys numbered 1302 to 1306 inclusive each with 1 cubic centimeter. Ten days after the vaccination the immunity of each animal was tested in the following manner: Two agar slant 48-hour cultures of "Pest Virulent" (from guinea pig number 1324, second transplant) were each suspended in 5 cubic centimeters of bouillon and animals numbered 1298 and 1300 to 1305, and controls numbered 1414 to 1422 were each inoculated subcutaneously with 0.25 cubic centimeter of this suspension, equal to ½ oese "Pest Virulent;" and animal number 1306, with 0.5 cubic centimeter, equal to 1 oese.

Animal No.	Vaccination October 25.	Reinoculated November 10.	Result.	Autopsy and remarks.
1298	2 agar slants "Pest Avirulent."	½ oese "Pest Virulent."	Dead Nov. 22 (12 days).	No pest bacilli in smears from spleen. Large suppurating bubo about point of inoculation containing pest bacilli and many cocci and other bacilli.
1299	do			Found dead about 12 hours after vaccination. There is a large suppurating wound over the abdomen. Cultures from this area developed colonies which were later identified as those of staphylococci and streptococci and of a colon bacillus. Cultures from the spleen show many staphylococci. Cultures near the point of inoculation show a few colonies of "Pest Avirulent" which have evidently survived.
1300	do	½ oese "Pest Virulent."	Alive and well.	
1301	do	do	do	
1302	1 agar slant "Pest Avirulent."	do	do	
1303	do	do	Dead Nov. 20 (10 days).	No bacilli found in smears from spleen. Suppurating bubo about point of inoculation containing pest bacilli.
1304	do	do	Dead Nov. 16 (6 days).	Very numerous pest bacilli in smears from spleen.
1305	do	do	Dead Nov. 17 (7 days).	No organisms found in smears from spleen. Cultures from the heart, negative. Bubo at point of inoculation comparatively small. A number of pest bacilli present.
1306	do	1 oese "Pest Virulent."	Alive and well.	

SERIES 12—Continued.

CONTROLS.				
Anmial No.	Vaccination October 25.	Reinoculated November 10.	Result.	Autopsy and remarks.
1414	None	½ oese "Pest Virulent."	Dead Nov. 20 (10 days).	Few pest bacilli in smears from spleen. Chronic bubo.
1415	do	do	Dead Nov. 13 (3 days).	Innumerable pest bacilli in smears from spleen.
1416	do	do	Dead Nov. 14 (4 days).	Countless pest bacilli in smears from spleen.
1417	do	do	do	Do.
1418	do	do	Dead Nov. 15 (5 days).	Do.
1419	do	do	Dead Nov. 14 (4 days).	Do.
1420	do	do	Dead Nov. 13 (3 days).	Only comparatively few pest bacilli in smears from spleen. Œdematous infiltration and countless numbers near point of inoculation.
1421	do	do	do	Very numerous pest bacilli in smears from spleen.
1422	do	do	Dead Nov. 14.	Countless pest bacilli in smears from spleen. Hæmorrhagic infarction of the intestine.

SERIES 18.—*Inoculations with "Pest Avirulent" in monkeys.*

On November 6, twenty 48-hour agar cultures of "Pest Avirulent" were suspended in 20 cubic centimeters of bouillon and each animal was vaccinated with 2 cubic centimeters of this suspension, equal to 2 agar slant cultures. On November 23, seventeen days after the first inoculation, two 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1452, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was reinoculated subcutaneously, on the opposite side of the body to that upon which the first inoculation was performed, with 0.25 cubic centimeter of this suspension, equal to ½ oese. Ten control animals were also inoculated at the same time with the same dose.

Animal No.	Vaccinated November 6 subcutaneously with—	Reinoculated November 23 with—	Result.	Autopsy and remarks.
1373	2 agar cultures "Pest Avirulent."	½ oese "Pest Virulent."	Dead Nov. 29, after 6 days.	A few typical pest bacilli in smears from spleen.
1374	do	do	Alive and well	
1375	do	do	do	
1376	do	do	do	
1377	do	do	Dead Nov. 29, after 6 days.	Pest bacilli very numerous in smears from spleen.
1378	do	do	Alive and well	

SERIES 18—Continued.

Animal No.	Vaccinated November 6 subcutaneously with—	Reinoculated November 23 with—	Result.	Autopsy and remarks.
1379	2 agar cultures "Pest Avirulent."	½ oese "Pest Virulent."	Dead Dec. 6, after 13 days.	A large sloughing ulcer 4 centimeters in diameter about point of inoculation. No plague bacilli in cultures and smears from the spleen. Many bacilli and cocci in smears from the ulcer. The animal has evidently died of plague intoxication, the organisms multiplying locally.
1380	do	do	Dead Nov. 30, after 7 days.	A fair number of pest bacilli in smears from spleen.
1381	do	do	Dead Dec. 2, after 9 days.	Only two involution forms of organism found in smears from spleen; there is a bubo about the point of inoculation in smears from which a number of pest bacilli are present.
1382	do	do	Alive and well.	

CONTROLS NUMBERS 1484 TO 1494.

These controls constitute the same animals given in Series 17. They all died of acute pest infection in from four to five days. For details of the autopsies see Series 17 (p. 213).

SERIES 51.—*Inoculations with "Pest Avirulent" in monkeys.*

Twenty-five 48-hour agar slant cultures of the strain "Pest Avirulent" were suspended in 25 cubic centimeters of saline solution (1 cubic centimeter to each culture). Monkeys numbered 2706 to 2712 each received subcutaneously 2 cubic centimeters of this suspension, equal to 2 agar cultures, and monkeys numbered 2713 to 2720 each received 1 cubic centimeter equal to 1 agar culture. On November 28, one month after the vaccination, the immunity of the animals was tested with a suspension of the strain "Pest Virulent" in exactly the same manner as was the immunity of the animals comprising Series 48 and 52 (p. 217). Twenty-five control animals, numbered 2896 to 2920, were also inoculated on this date and with the same suspension. They all died of acute pest infection. For the details of the protocols see Series 48 (p. 195).

Animal No.	Vaccinated October 29 subcutaneously with—	Reinoculated November 28 subcutaneously with—	Result.	Autopsy and remarks.
2706	Two 48-hour agar cultures "Pest Avirulent."	¾ oese "Pest Virulent."	Dead Dec. 1, after 3 days.	Few pest bacilli in smears from spleen. Numerous at point of inoculation.
2707	do	do	Alive and well.	
2708	do	do	do	
2709	do	do	Dead Dec. 4, after 6 days	Buboes with few pest bacilli.
2710	do	do	Alive and well.	
2711	do	do	do	
2712	do	do	do	

SERIES 51—Continued.

Animal No.	Vaccinated October 29 subcutaneously with—	Renoculated November 28 subcutaneously with—	Result.	Autopsy and remarks.
2713	One 48-hour agar culture "Pest Avirulent."	½ oese "Pest Virulent."	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2714	do	do	Dead Dec. 2, after 4 days.	Do.
2715	do	do	Dead Dec. 3, after 5 days.	Bubo and numerous pest bacilli in smears from spleen.
2716	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2717	do	do	Alive and well	
2718	do	do	do	
2719	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2720	do	do	Alive and well	

SERIES 32.—*Inoculations with "Pest Avirulent" in guinea pigs.*

May 16: Twenty-four 48-hour agar slant cultures of "Pest Avirulent" were suspended in 24 cubic centimeters of saline solution (1 cubic centimeter to each culture). Animals numbered 1975 to 1986, except number 1979, were each inoculated intraperitoneally with 2 cubic centimeters, equal to 2 agar cultures. The guinea pigs comprising this series were small, averaging from 150 to 175 grams in weight. On July 16, two months after the vaccination, the animals which remained alive were all reinoculated in the following manner: A 48-hour agar culture of "Pest Virulent" (from guinea pig number 2174, second transplant) was suspended in 5 cubic centimeters of bouillon and 5 oesen of this suspension rubbed over a small, freshly shaved area on the abdomen and the skin scarified with a knife. Twenty-five controls were inoculated in the same manner and served for this series and for the following one. (Series 37).

Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
1975	Two 48-hour cultures "Pest Avirulent."	5 oesen suspension "Pest Virulent."	Alive and well	
1976	do	do	do	
1977	do	do	do	
1978	do	do	Dead July 21, after 5 days.	Numerous pest bacilli in smears from spleen. Many swollen. Few postmortem bacilli.
1980	do	do	Alive and well	

SERIES 32—Continued.

Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
1981	Two 48-hour cultures "Pest Avirulent."		Not reinoculated; dead May 19, 3 days after vaccination.	No evidence of pest at the postmortem examination. Peritoneum hyperæmic but no hæmorrhages. In smears from spleen no organisms seen. Cultures from spleen and heart negative. In cultures from the abdominal cavity about 10 colonies developed. Spleen not swollen.
1982	do		Not reinoculated; dead May 17, 23 hours after vaccination.	No evidence of pest. Marked hyperæmia of the abdominal wall. Culture from the heart negative. Culture from the abdominal cavity, fair number of pest bacilli.
1983	do	5 oesen suspension "Pest Virulent."	Alive and well	
1984	do		Not reinoculated; dead May 17, less than 24 hours after vaccination.	No evidence of pest infection. No hæmorrhages in abdomen. Walls slightly œdematous. Culture from the heart negative. From abdominal cavity numerous colonies of pest. 1 cc. of the fluid present in the abdominal cavity was injected into the abdomen of guinea pig No. 1998 which remained alive.
1985	do		Not reinoculated; dead May 22, 6 days after vaccination.	No evidences of pest. Abdominal wall hyperæmic. No hæmorrhage. Spleen not swollen. No fluid in abdomen. In cover slip from the spleen and abdomen no bacilli seen. Culture from abdomen 15 colonies of pest bacilli. In culture from heart, 2 colonies.
1986	do		Not reinoculated; dead May 20, 4 days after vaccination.	Culture from heart negative. Culture from the abdomen, numerous colonies with typical morphology of pest bacillus.

SERIES 32—Continued.

Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
		CONTROLS.		
2008		5 oesen suspension "Pest Virulent."	Dead July 21, after 5 days.	Pest. Numerous bacilli in smears from the spleen.
2009		do	Dead July 26, after 10 days.	Do.
2010		do	do	Do.
2011		do	Dead July 23, after 7 days.	Do.
2012		do	Dead July 21, after 5 days.	Do.
2013		do	Dead July 23, after 7 days.	Do.
2014		do	Dead July 19, after 2½ days.	Do.
2015		do	Dead July 26, after 10 days.	Do.
2016		do	Dead July 20, after 4 days.	Do.
2017		do	Dead July 23, after 7 days.	Do.
2018		do	do	Do.
2019		do	do	Do.
2020		do	do	Do.
2021		do	Dead July 20, after 4 days.	Do.
2022		do	Dead July 24, after 8 days.	Do.
2023		do	Dead July 22, after 6 days.	Do.
2024		do	Dead July 23, after 7 days.	Do.
2025		do	do	Do.
2026		do	do	Do.
2027		do	do	Do.
2028		do	do	Do.
2029		do	Dead July 22, after 6 days.	Do.
2030		do	do	Do.
2031		do	Dead July 28, after 7 days.	Do.
2032		do	do	Do.

SERIES 37.—*Inoculations with "Pest Avirulent" in guinea pigs.*

May 20: Ten 24-hour agar cultures of "Pest Avirulent" were suspended in 10 cubic centimeters of normal saline solution (1 cubic centimeter to each culture). Each animal received 1 cubic centimeter intraperitoneally. Some of the guinea pigs of this series did not weigh over 175 grams. One of these apparently succumbed from the effect of the vaccination. On July 16, nearly two months after the vaccination, the animals were all reinoculated with 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2174, second transplant in 5 cubic centimeters of bouillon), rubbed over a freshly shaved area on the abdomen which was scarified with a scalpel. These animals were all inoculated in exactly the same manner and on the same day as the animals comprising Series 32, 33 and 38 (pp. 218 and 219). Twenty-five control animals, numbers 2098 to 2122, were also inoculated, all of which died. The details of the autopsies are given in Series 32 (p. 206).

Animal No.	Immunized May 20 intraperitoneally with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
2036	One 24-hour culture "Pest Avirulent."	----------	Died June 1, 12 days after vaccination.	No pest.
2037	----do----------	5 oesen suspension "Pest Virulent" over abdomen.	Alive and well ---	
2038	----do----------	----do----------	Dead July 26, after 10 days.	Typical pest spleen and bubo. Numerous pest bacilli in smears from spleen.
2039	----do----------	----do----------	Alive and well ---	
2040	----do----------	----do----------	----do----------	
2041	----do----------	----do----------	----do----------	
2042	----do----------	----do----------	----do----------	
2043	----do----------	----------	Small guinea pig, 180 grams, died May 21, less than 24 hours after vaccination.	No post-mortem evidence of pest. No peritonitis or hæmorrhages. Culture from the abdomen developed from 150 to 200 colonies. Evidently the majority of the organisms had perished. Culture from heart sterile.
2044	----do----------	5 oesen suspension "Pest Virulent" over abdomen.	Alive and well ---	

SERIES 39.—*Inoculations with "Pest Avirulent" in guinea pigs.*

June 10: Fifteen 24-hour agar slant cultures of "Pest Avirulent" were suspended in 15 cubic centimeters of normal saline solution, and each guinea pig was inoculated subcutaneously with 1 cubic centimeter of the suspension. On August 3, nearly two months after the vaccination, the animals were all reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2204, second transplant, in 5 cubic centimeters bouillon) being rubbed over a shaved area on the abdomen which was scarified with a knife. Twenty control guinea pigs were also inoculated at the same time and in the same manner. These animals also served for controls for series number 40 (p. 219).

Animal No.	Inoculated subcutaneously June 10 with—	Reinoculated August 3 with—	Result.	Autopsy and remarks.
2075	One 24-hour agar culture "Pest Avirulent."	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Dead Aug. 11, after 8 days	Pest.
2076	do	do	Dead Aug. 12, after 9 days	Do.
2077	do	do	Alive and well	
2078	do	do	do	
2079	do	do	Dead Aug. 10, after 7 days	Typical pest bubo. Innumerable bacilli.
2080	do	do	Alive and well	
2081	do	do	Dead Aug. 8, after 5 days	Innumerable pest bacilli in smears from the spleen.
2082	do	do	Dead Aug. 11, after 8 days	Do.
2083	do	do	Alive and well	
2084	do	do	Dead Aug. 12, after 9 days	Pest.
2085	do	do	Alive and well	
2086	do	do	do	
2087*	do	do	do	
2088	do	do	do	
2089	do	do	Dead Aug. 14, after 11 days	Pest. Typical spleen, containing necrotic foci.

SERIES 39—Continued.

CONTROLS.				
Animal No.	Inoculated subcutaneously June 10 with—	Reinoculated August 3 with—	Result.	Autopsy and remarks.
2249	------	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Dead Aug. 12, after 9 days	Innumerable pest bacilli in smears from spleen.
2250	------	------do------	Dead Aug. 11, after 8 days	Do.
2251	------	------do------	Dead Aug. 13, after 10 days	Do.
2252	------	------do------	Dead Aug. 10, after 7 days	Do.
2253	------	------do------	Dead Aug. 14, after 11 days	Do.
2254	------	------do------	Dead Aug. 8, after 5 days	Do.
2255	------	------do------	Dead Aug. 11, after 8 days	Do.
2256	------	------do------	Dead Aug. 8, after 5 days	Do.
2257	------	------do------	Dead Aug. 14, after 11 days	Do.
2258	------	------do------	Dead Aug. 11, after 8 days	Do.
2259	------	------do------	Dead Aug. 7, after 4 days	Do.
2260	------	------do------	Dead Aug. 10, after 7 days	Do.
2261	------	------do------	Dead Aug. 9, after 6 days	Do.
2262	------	------do------	Dead Aug. 13, after 10 days	Do.
2263	------	------do------	------do------	Do.
2264	------	------do------	Dead Aug. 11, after 8 days	Do.
2265	------	------do------	------do------	Do.
2266	------	------do------	Dead Aug. 10, after 7 days	Do.
2267	------	------do------	Dead Aug. 9, after 6 days	Do.
2268	------	------do------	Dead Aug. 13, after 10 days	Do.

* Animal was pregnant at the time of vaccination and gave birth to two healthy young 7 days after its reinoculation.

SERIES 41.—*Inoculations with "Pest Avirulent" in guinea pigs.*

June 30: Twenty-one 24-hour agar slant cultures of "Pest Avirulent" were suspended in 21 cubic centimeters of saline solution and each guinea pig was inoculated subcutaneously with 1 cubic centimeter of this suspension. On August 28, two months after the vaccination, the animals were all reinoculated with 5 oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2315, second generation, in 10 cubic centimeters bouillon) being rubbed over a freshly shaved and scarified area on the abdomen. Twenty-five control animals were also inoculated at the same time and in the same manner.

Animal No.	Vaccinated June 30 subcutaneously with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2146	One 24-hour culture "Pest Avirulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2147	-----do------	-----do------	Dead Sept. 4, after 7 days	Pest.
2148	-----do------	-----do------	Dead Sept. 6, after 9 days	Moderate-sized bubo and abscesses in the spleen.
2149	-----do------	-----do------	Alive and well	
2150	-----do------	-----do------	-----do------	

SERIES 41—Continued.

Animal No.	Vaccinated June 30 subcutaneously with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2151	One 24-hour culture "Pest Avirulent."	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Alive and well	
2152	do	do	do	
2153	do	do	do	
2154	do	do	do	
2155	do	do	Dead Sept. 7, after 10 days	Large bubo. Miliary abscesses in spleen.
2156	do	do	Alive and well	
2157	do	do	do	
2158	do	do	do	
2159	do	do	do	
2160	do	do	Dead Sept. 12, after 15 days	Pest bubo. Very few bacilli in the pus from the bubo.
2161	do	do	Alive and well	
2162	do	do	do	
2263	do	do	Dead Sept. 7, after 10 days	Bubo. Spleen apparently normal. No bacilli in smears.
2164	do	do	Alive and well	
2165	do	do	Dead Sept. 7, after 10 days	Large bubo. Abscess in the spleen.
2166	do	do	Alive and well	
		CONTROLS.		
2362		5 oesen suspension "Pest Virulent rubbed over abdomen.	Dead Sept. 4, after 7 days	Numerous pest bacilli in smears from spleen.
2363		do	do	Do.
2364		do	do	Do.
2365		do	do	Do.
2366		do	Dead Sept. 2, after 5 days	Do.
2367		do	Dead Sept. 4, after 7 days	Do.
2368		do	Dead Sept. 2, after 5 days	Do.
2369		do	do	Do.
2370		do	do	Do.
2371		do	Dead Sept. 4, after 7 days	Do.
2372		do	Dead Sept. 2, after 5 days	Do.
2373		do	Dead Sept. 12, after 15 days	Do.
2374		do	Dead Sept. 2, after 5 days	Do.
2375		do	Dead Sept. 8, after 11 days	Do.
2376		do	Dead Sept. 4, after 7 days	Do.
2377		do	Dead Sept. 6, after 9 days	Do.
2378		do	Dead Sept. 2, after 5 days	Do.
2379		do	Dead Sept. 4, after 7 days	Do.
2380		do	Dead Sept. 6, after 9 days	Do.
2381		do	Dead Sept. 2, after 5 days	Do
2382		do	Dead Sept. 4, after 7 days	Do.
2383		do	do	Do.
2384		do	do	Do.
2385		do	Dead Sept. 6, after 9 days	Do.
2386		do	Dead Sept. 8, after 11 days	Do.

SERIES 46.—*Inoculations with "Pest Avirulent" in guinea pigs.*

October 26: Fifteen 48-hour agar slant cultures of the strain "Pest Avirulent" were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). Each guinea pig of the series was inoculated subcutaneously with 1 cubic centimeter of this suspension. On November 26, one month after the vaccination, the immunity of the animals was tested in the following manner:

Five oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2858, second transplant in 10 cubic centimeters of bouillon) being rubbed over a freshly shaved but not scarified area of the abdomen. Twenty-five control animals were also inoculated at the same time and in the same manner. The immunity of the animals of this series was tested on the same date and with exactly the same suspension as was that of the animals comprising Series 47, 50, and 54. (See pp. 220, 197, and 232.)

Animal No.	Vaccinated October 26 subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2633	One 24-hour culture "Pest Avirulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical pest buboes and spleen.
2634	do	do	Alive and well	
2635	do	do	Dead Dec. 8, after 12 days	Old bubo and few abscesses in spleen.
2636	do	do	Alive and well	
2637	do	do	do	
2638	do	do	do	
2639	do	do	do	
2640	do	do	do	
2641	do	do	Dead Dec. 3, after 7 days	Large bubo. Spleen contains few abscesses.
2642	do	do	Alive and well	
2643	do	do	do	
2644	do	do	do	
2645	do	do	do	
2646	do	do	do	
2647	do	do	do	
		CONTROLS.		
2870		5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 3, after 7 days	Typical bubo. Numerous pest bacilli in smears from spleen.
2871		do	Dead Dec. 1, after 5 days	Do.
2872		do	Dead Dec. 3, after 7 days	Do.
2873		do	Dead Dec. 2, after 6 days	Do.
2874		do	Dead Dec. 4, after 8 days	Do.
2875		do	Dead Dec. 1, after 5 days	Do.
2876		do	Dead Nov. 30, after 4 days	Do.
2877		do	Dead Dec. 2, after 6 days	Do.
2878		do	Dead Nov. 30, after 4 days	Do.
2879		do	Dead Dec. 5, after 9 days	Do.

SERIES 46.—Continued.

CONTROLS—Continued.				
Animal No.	Vaccinated October 26 subcutaneonsly with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2880		5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical bubo. Numerous pest bacilli in smears from spleen.
2881		do	Dead Dec. 7, after 11 days	Do.
2882		do	Dead Nov. 30, after 4 days	Do.
2883		do	do	Do.
2884		do	Dead Dec. 2, after 6 days	Do.
2885		do	Dead Dec. 1, after 5 days	Do.
2886		do	Dead Nov. 30, after 4 days	Do.
2887		do	Dead Dec. 1, after 5 days	Do.
2888		do	do	Do.
2889		do	Dead Nov. 30, after 4 days	Do.
2890		do	Dead Dec. 1, after 5 days	Do.
2891		do	Dead Nov. 30, after 4 days	Do.
2892		do	Dead Dec. 3, after 7 days	Do.
2893		do	Dead Dec. 1, after 5 days	
2894		do	do	

SERIES 17.—*Inoculations with the strain "Maassen Alt" in monkeys.*

November 3: Six 48-hour agar slant cultures of "Maassen Alt" were suspended in 7 cubic centimeters of bouillon. Animals numbered 1369 to 1371 were each inoculated subcutaneously with 2 cubic centimeters of this suspension which equals nearly 2 agar slants, and animal number 1372 with 1 cubic centimeter, or nearly 1 agar slant of "Maassen Alt." On November 23, twenty days after the vaccination, 2 agar slant cultures of "Pest Virulent" (from guinea pig number 1452, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was reinoculated subcutaneously, on the opposite side of the body to that upon which previous inoculation had been performed, with 0.25 cubic centimeter of this suspension, which is equal to $\frac{1}{2}$ oese "Pest Virulent." Eleven control animals were at the same time also inoculated subcutaneously with the same dose.

Animal No.	Vaccinated November 3 subcutaneously with—	Infected November 23 with—	Result.	Autopsy and remarks.
1369	Nearly 2 agar slants "Maassen Alt."	$\frac{1}{2}$ oese "Pest Virulent."	Alive and well	
1370	do	do	do	
1371	do	do	do	
1372	Nearly 1 agar slant "Maassen Alt."	do	Dead Nov. 29, after 6 days.	Innumerable pest bacilli in smears from the spleen.

SERIES 17—Continued.

CONTROLS.				
Animal No.	Vaccinated November 3 subcutaneously with—	Infected November 23 with—	Result.	Autopsy and remarks.
1484	----------	⅛ oese "Pest Virulent."	Dead Nov. 28, after 5 days.	Innumerable pest bacilli in smears from the spleen.
1485	----------	do	do	Do.
1486	----------	do	do	Do.
1487	----------	do	Dead Nov. 27, after 4 days.	Do.
1488	----------	do	do	Do.
1489	----------	do	do	Do.
1490	----------	do	Dead Nov. 28, after 5 days.	Do.
1491	----------	do	Dead Nov. 27, after 4 days.	Do.
1492	----------	do	do	Do.
1493	----------	do	do	Do.
1494	----------	do	do	Do.

SERIES 21.—*Inoculations with the strain "Maassen Alt" in monkeys.*

November 14: Twenty-one 48-hour agar slant cultures of "Maassen Alt" were suspended in 21 cubic centimeters of bouillon. Monkeys numbered 1438 to 1445 inclusive each received 2 cubic centimeters subcutaneously, which is equal to 2 agar slant cultures, and monkeys numbered 1446 to 1449 inclusive, 1 cubic centimeter subcutaneously, equal to 1 agar slant culture. On November 28, two weeks after the vaccination, the animals were all reinoculated in the following manner. Two agar slant cultures of "Pest Virulent" (from guinea pig number 1479, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was inoculated subcutaneously on the opposite side of the body to which the vaccination had been made with 0.25 cubic centimeter of this suspension, equal to ½ oese "Pest Virulent." Twelve control animals were all inoculated at the same time with the same dose of this suspension, except animals numbered 1503 and 1504, which were very large monkeys and which received 0.33 cubic centimeter.

Animal No.	Vaccinated November 14 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
1438	2 agar slant cultures.	½ oese "Pest Virulent."	Alive and well.	
1439	do	do	Dead Dec. 5, after 7 days.	Very few pest bacilli in smears from the spleen and near point of inoculation. Tissues near point of inoculation reddened and œdematous.
1440	do	do	Alive and well.	
1441	do	do	do	
1442	do	do	Dead Dec. 6, after 8 days.	A few bacilli in smears from the spleen. Innumerable in ulcerating bubo which also contains numerous cocci.

SERIES 21—Continued.

Animal No.	Vaccinated November 14 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
1443	2 agar slant cultures.	½ oese "Pest Virulent."	Alive and well.	
1444	do	do	do	
1445	do	do	do	
1446	1 agar slant culture.	do	do	
1447	do	do	Dead Dec. 4, after 6 days.	Pest bacilli fairly numerous in smears from the spleen. No appearance of bubo about point of inoculation although pest bacilli are numerous in the tissues in this region.
1448	do	do	Dead Dec. 18, after 10 days.	A large sloughing abscess at the point of inoculation in which a fair number of pest bacilli and streptococci are present. Smears from spleen show a few bacilli.
1449	do	do	Alive and well.	
		CONTROLS.		
1499		½ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1500		do	Dead Dec. 3, after 5 days.	Do.
1501		do	Dead Dec. 1, after 3 days.	No bacilli found in one smear from spleen. At point of inoculation a hæmorrhagic bubo is present in which numerous pest bacilli are present.
1502		do	do	Very numerous pest bacilli in smears from spleen.
1503		⅔ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
1504		do	Dead Dec. 4, after 6 days.	Do.
1505		½ oese "Pest Virulent."	Dead Dec. 3, after 5 days.	Fairly numerous pest bacilli in smears from spleen.
1506		do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
1507		do	Dead Dec. 3, after 5 days.	Fairly numerous pest bacilli in smears from spleen.
1508		do	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
1509		do	do	Do.
1510		do	Dead Dec. 6 after 8 days.	Very numerous pest bacilli in smears from spleen.

SERIES 24.—*Inoculations with the strain "Maassen Alt" in monkeys.*

On December 4, thirty-two, 48-hour agar slant cultures of "Maassen Alt" were suspended in 32 cubic centimeters of bouillon (1 cubic centimeter to each tube). Monkeys numbered 1536 to 1547 inclusive were inoculated subcutaneously each with 2 cubic centimeters of this suspension, equal to 2 agar cultures; monkeys numbered 1548 to 1552 inclusive with 1 cubic centimeter each and monkey number 1553 with a little less than ½ cubic centimeter, equal to about ½ culture. Four of the animals succumbed, apparently from the result of the vaccination. On December 18 the surviving ones were all reinoculated in the following manner: A 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1516, second transplant) was suspended in 5 cubic centimeters of bouillon, and each animal inoculated subcutaneously on the opposite side of the body to that upon which the first inoculation was given with 0.2 cubic centimeter, which equals a little less than ½ oese of "Pest Virulent." Attention must be called to the fact that this series was inoculated exactly the same as Series 23. Among the animals comprising the present Series 24 there were also a number of large monkeys. For controls, the same animals given as controls in Series 23 (see p. 191), numbered 1594 to 1605, were employed, all being inoculated in the same manner and on the same day. It will be seen from the table that a number of the controls did not die and hence conclusions regarding the exact value of the immunization in this series can not be drawn.

Animal No.	Vaccinated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1536	2 agar slant cultures, "Maassen Alt."	----------	Dead Dec. 7, 3 days after first inoculation.*	Skin burned and incised at point of inoculation revealing an abscess containing pus in which numerous organisms are present. Smears from spleen are negative. Cultures from the spleen developed one colony of *Staphylococcus aureus*. Culture from abscess shows very numerous colonies of *Staphylococcus aureus* and also of "Maassen Alt."
*1537	-----do-----	----------	Dead Dec. 5, one day after first inoculation.*	Cultures from the point of inoculation show few colonies of "Maassen Alt" and of *Staphylococcus* aureus. Culture from the spleen negative.
1538	-----do-----	Nearly ½ oese "Pest Virulent."	Alive and well.	

*It is not entirely clear that these animals succumbed to a pest infection alone. They were evidently in poor health and in three the *Staphylococcus aureus* was also isolated. In the case of monkey number 1552 it seems clear, however, that the animal really succumbed to pest infection. It must be noted that animals numbered 1536, 1537 and 1547 died in a much shorter time than one would expect from a pest infection with an avirulent organism.

SERIES 24—Continued.

Animal No.	Vaccinated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1539	2 agar slant cultures, "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive and well.	
1540	do	do	do	
1541	do	do	do	
1542	do	do	do	
1543	do	do	do	
1544	do	do	do	
1545	do	do	Dead Dec. 21, after 3 days.	Animal considerably emaciated. No organisms found in spleen. Cultures and smears from the tissues near the point of inoculation show numerous pest bacilli.
1546	do	do	Alive and well.	
*1547	do		Died from first inoculation Dec. 7, after 3 days.*	Skin burned and incised, revealing a small abscess in which numerous pest bacilli ("Maassen Alt") are present. Cultures developed numerous colonies of this organism and of *Staphylococcus aureus*.
1548	1 agar slant culture, "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive	
1549	do	do	do	
1550	do	do	do	
1551	do	do	do	
*1552	do		Died from first inoculation Dec. 10, after 6 days.*	Nothing apparently abnormal about the point of inoculation. No abscess or pest bacilli seen in smears. In 2 cultures from the spleen, one developed 12 colonies and the other 14 colonies of "Maassen Alt."
1553	About ½ agar slant culture "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive	

*It is not entirely clear that these animals succumbed to a pest infection alone. They were evidently in poor health and in three the *Staphylococcus aureus* was also isolated. In the case of monkey number 1552 it seems clear, however, that the animal really succumbed to pest infection. It must be noted that animals numbered 1536, 1537 and 1547 died in a much shorter time than one would expect from a pest infection with an avirulent organism.

SERIES 52.—*Inoculations with the strain "Maassen Alt" in monkeys.*

Twenty-two 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 22 cubic centimeters saline solution (1 cubic centimeter to each culture). Monkeys numbered 2721 to 2728 received subcutaneously each 2 cubic centimeters, equal to 2 agar slant cultures. Monkeys numbered 2729 to 2735 each received 1 cubic centimeter, equal to 1 agar slant culture. On November 28, one month after the vaccination, the immunity of the animals was tested by the subcutaneous injection of the strain "Pest Virulent" in exactly the same manner as was the immunity of the animals in Series 48 and 51. (See pp. 195 and 203.) Twenty-five control animals, numbered 2896 to 2920, were also inoculated in the same manner with the same suspension and succumbed to acute infection. For the details of the protocols see Series 48.

Animal No.	Vaccinated October 26 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
2721	2 agar cultures strain "Maassen Alt."	$\frac{1}{2}$ oese strain "Pest Virulent."	Alive and well.	
2722	do	do	Dead Nov. 8, after vaccination.	No evidence of bubo or inflammation at point of inoculation. Cover slips no excess of leucocytes, no bacteria. Cultures from spleen and point of inoculation negative. Three cultures. Large amounts material inoculated.
2723	do	$\frac{1}{2}$ oese strain "Pest Virulent."	Alive and well.	
2724	do	do	Dead Dec. 4, after 6 days.	Numerous pest bacilli in smears from spleen.
2725	do	do	do	Bubo with numerous pest bacilli.
2726	do	do	Alive and well.	
2727	do	do	do	
2728	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2729	1 agar culture strain "Maassen Alt."	do	Alive and well.	
2730	do	do	Dead Dec. 3, after 5 days.	Bubo. Numerous pest bacilli in smears from spleen.
2731	do	do	Alive and well.	
2732	do	do	do	
2733	do	do	Dead Dec. 3, after 5 days.	Bubo. Few pest bacilli.
2734	do	do	Dead Dec. 1, after 3 days.	Innumerable pest bacilli in smears from spleen.
2735	do	do	Dead Dec. 2, after 2 days.	Bubo. Fair numbers of pest bacilli in smears from spleen.

SERIES 33.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

May 16: Twenty-two 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 22 cubic centimeters of saline solution (1 cubic centimeter to each culture). Guinea pigs numbered 1987 to 1996 were inoculated intraperitoneally each with 1 cubic centimeter. On July 16, two months after the vaccination, the animals were all reinoculated with the strain "Pest Virulent" in exactly the same manner as the animals in Series 32, 37 and 38 (see pp. 204, 207 and 219), the same 25 control animals, numbered 2098 to 2122, all of which died of pest, serving for all three of these series.

Animal No.	Vaccinated intraperitoneally May 16 with—	Infected July 16 with—	Result.	Autopsy and remarks.
1987	1 cc. "Maassen Alt."	5 oesen "Pest Virulent rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	
1988	do	do	do	
1989	do	do	do	
1990	do	do	do	
1991	do	do	do	
1992	do		Dead May 17, about 21 hours after vaccination.	A few hæmorrhages in the abdominal walls. No fluid. Culture from the abdominal cavity shows a fair number of pest bacilli.
1993	do	5 oesen "Pest Virulent" rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	
1994	do		Dead May 17, 23 hours after vaccination.	No evidences of pest infection. Little fluid in the abdominal cavity, culture from heart negative. In culture from abdominal cavity a fair number of pest bacilli developed.
1995	do		Dead May 18, 2 days after vaccination.	Cultures from heart sterile. Culture from abdomen, a rich growth of pest.
1996	do	5 oesen "Pest Virulent" rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	

SERIES 38.—*Inoculation with the strain "Maassen Alt" in guinea pigs.*

May 20: Eight 24-agar slant cultures of "Maassen Alt" were suspended in 8 cubic centimeters of normal saline solution (1 cubic centimeter to each culture) and each animal was inoculated intraperitoneally with 1 cubic centimeter of the suspension. One animal succumbed from the effect of the vaccination. On July 16, about two months after the vaccination, the animals were all reinoculated, 5 oesen of the same suspension of "Pest Virulent" employed in reinoculating Series 32, 33 and 37 being rubbed over a shaved and scarified area on the abdomen. The same twenty-five control animals, numbered 2098 to 2122, employed in Series 32 and 33 (see pp. 204 and 218), which all died, answered for controls for the present series.

Animal No.	Vaccinated May 20, intraperitoneally with—	Infected July 16 with—	Result.	Autopsy and remarks.
2045	One 24-hour culture "Maassen Alt."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2046	do	do	Dead July 31, after 15 days.	Very few pest bacilli in smears from the spleen. Small bubo.
2047	do	do	Alive and well.	
2048	do	do	do	
2049	do	do	do	
2050	do		Dead May 22, 2 days after vaccination.	Culture from heart and spleen negative. From the abdomen about 100 colonies of "Maassen Alt."
2051	do	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead July 21, after 5 days.	Fair number of pest bacilli and numerous post-mortem invading bacilli in smears from spleen.

SERIES 40.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

June 15: Fifteen 24-agar slant cultures of "Maassen Alt" were suspended in 15 cubic centimeters saline solution, and each guinea pig inoculated subcutaneously with 1 cubic centimeter of this suspension. On August 3, nearly one and one-half months after the vaccination, the animals were all but one reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2204, second transplant, in 5 cubic centimeters bouillon) being rubbed over a shaved area on the abdomen which was lightly scarified with a knife. It is to be noticed that these animals were all inoculated in exactly the same manner as those described in Series 39. The same twenty control animals, numbered 2249 to 2268 (see p. 209), which all died, also served for the present series.

SERIES 40—Continued.

Animal No.	Vaccinated subcutaneously June 15 with—	Infected August 3 with—	Result.	Autopsy and remarks.
2125	One 24-hour culture "Maassen Alt."	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2126	do	do	do	
2127	do	do	do	
2128	do		Dead June 16, less than 24 hours after vaccination.	Culture from heart and spleen negative. Cover slip from point of inoculation shows a few degenerating bacilli. Animal evidently died of plague toxæmia.
2129	do	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2130	do	do	do	
2131	do	do	Dead Aug. 14, after 11 days.	Few pest bacilli in smears from spleen.
2132	do	do	Alive and well	
2133	do	do	Dead Aug. 12, after 9 days.	Pest.
2134	do	do	Alive and well	
2135	do	do	do	
2136	do	do	do	
2137	do	do	do	
2138	do	do	Dead Aug. 13, after 10 days.	Pest.
2139	do	do	Alive and well	

SERIES 47.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

October 26: Fifteen 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). Each guinea pig of the series was inoculated subcutaneously with 1 cubic centimeter of the suspension equal to 1 agar slant culture. On November 26, about one month after the vaccination, the immunity of the animals was tested in the following manner:

Five oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2858, second transplant, in 10 cubic centimeters of bouillon) being rubbed over the freshly shaved but not scarified area of the abdomen. Twenty-five control animals, numbered 2870 to 2894, were also inoculated with the virulent organism at the same time and in the same manner. All of these succumbed to acute plague infection. For the details of the protocols see Series 46 (p. 211).

SERIES 17—Continued.

The immunity of the animals in this series was tested in the same manner and with exactly the same suspension as was used for the animals comprising Series 46, 50, and 54. (See pp. 211, 197, and 232.)

Animal No.	Vaccinated October 26 subcutaneously with—	Infected November 26 with—	Result.	Autopsy and remarks.
2648	One 24-hour agar culture "Massen Alt."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2649	do	do	do	
2650	do	do	do	
2651	do	do	do	
2652	do	do	do	
2653	do		Died Oct. 27, 1 day after vaccination.	No plague bacilli found in cover slips from abdominal cavity or from point of inoculation. Some fluid had entered abdominal cavity. Animal evidently died of toxæmia.
2654	do	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2655	do	do	do	
2656	do	do	do	
2657	do	do	do	
2658	do	do	do	
2659	do	do	do	
2660	do	do	do	
2661	do	do	do	
2662	do	do	do	

EXPERIMENTS IN THE INOCULATION OF ANIMALS WITH EXTRACTS OF THE STRAIN "PEST VIRULENT."

SERIES 7.—*Inoculations in monkeys with extracts of the strain "Pest Virulent" (free receptors).*

Twenty-one, 48-hour slant cultures of the strain "Pest Virulent" were suspended in 21 cubic centimeters of saline solution. The organisms were killed by heating for one hour at 60° C. and the suspension placed in the incubator at 37° C. for three days. At the end of this time it was filtered through a Berkefeld candle. The animals were inoculated subcutaneously with the filtrate as shown below. Ten days later their immunity was tested by sticking them with a 10 cubic centimeter syringe needle which had been dipped in a suspension of "Pest Virulent" (one 48-hour agar slant "Pest Virulent," guinea pig number 1243, second transplant) suspended in 5 cubic centimeters of bouillon.

SERIES 7—Continued.

Animal No.	How immunized.	How infected.	Result.	Autopsy and remarks.
1242	5 cc. autolytic product = 50 mg. of culture.	Stuck with infected 10 cc. syringe needle.	Dead after 7 days.	Innumerable pest bacilli in spleen.
1245	do	do	Dead after 6 days.	Pest septicæmia. Numerous organisms in the heart's blood.
1246	do	do	Dead after 11 days.	Numerous organisms in the spleen.
1247	4.8 cc. autolytic product = 48 mg. of culture.	do	Alive	
		CONTROLS.		
1293		Stuck with infected 10 cc. syringe needle.	Dead after 4 days.	Smears from spleen and heart negative for organisms. Culture from heart, *Staphylococcus aureus*. Pest organism probably still localized near point of inoculation.
1294		do	Dead after 7 days.	No pest bacilli found in the smears from the spleen. Very numerous in smears from the tissues near the point of inoculation.
1295		do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen. Cultures from heart show *B. pestis* (pure).

SERIES 26.—*Inoculations in monkeys with extracts of the strain "Pest Virulent" (free receptors).*

On December 8 sixteen 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1514, second generation) were suspended in 16 cubic centimeters of distilled water, the organisms killed by heating at 60° C. for one hour and the suspension then placed in the incubator at 37° C. for four days. At the end of this time it was filtered through a Berkefeld candle. The suspension both before and after filtration was sterile. On December 12 the animals of this series were inoculated subcutaneously with the filtrate as below. On December 26, fourteen days after the first inoculation, the animals were infected in the following manner: Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1592, second transplant) were suspended in 30 cubic centimeters of bouillon (5 cubic centimeters to each culture). Each animal was inoculated subcutaneously on the opposite side of the body with 0.33 cubic centimeters, equal to $\frac{2}{3}$ oese, of "Pest Virulent." Twelve controls, numbered 1613 to 1624, were all inoculated at the same time with the same dose. The details of the results with the controls may be seen in Table No. 25. (See p. 194.) All of them succumbed to acute pest infection.

SERIES 26—Continued.

Animal No.	Inoculated December 12 subcutaneously with—	Reinoculation December 26 with—	Result.	Autopsy and remarks.
1583	Free receptors extracted from 5 agar slant cultures "Pest Virulent."	⅔ oese "Pest Virulent.	Dead Jan. 13, after 17 days.	Few pest bacilli in smears from spleen. Innumerable organisms in a large bubo over which the skin is not yet broken.
1584	——do——	——do——	Alive and well.	
1585	Free receptors extracted from 2 agar slant culture "Pest Virulent."	——do——	Dead Jan. 2, after 7 days.	Numerous pest bacilli in smears from spleen.
1586	Free receptors extracted from 1 agar slant culture "Pest Virulent."	——do——	Dead Dec. 31, after 5 days.	Very numerous pest bacilli in smears from the spleen.
1587	——do——	——do——	Dead Jan. 1, after 6 days.	Very few pest bacilli in smears from the spleen. Organisms very numerous in smears from bubo.

SERIES 29.—*Inoculations with artificial pest aggressin in monkeys and guinea pigs.*

On January 27, large test tubes of agar were inoculated with first transplant cultures of "Pest Virulent" from guinea pigs numbered 1700, 1698, 1697 and 1676, all of which animals died between the 23d to the 25th of January. The cultures were placed at 30° C. for two days. The growth was then suspended in distilled water, 1 cubic centimeter being used for each culture containing about 30 milligrams of the growth. The whole was mixed and placed on an electrical shaking machine for five days. The suspension was then heated for one and one-half hours at a temperature of 44° to 45° C. and 5 per cent carbolic acid added in sufficient quantity to form a 0.5 per cent solution. After one and one-half days the suspension was thoroughly centrifuged for four hours at a velocity of 4,000 revolutions per minute and the clear fluid above pipetted and used for the inoculations on February 7 as given below. Cultures showed this fluid to be sterile. On February 24 and 25, nearly three weeks after the first inoculation, the animals were reinoculated in the following manner: In the case of the guinea pigs, the abdomen of each was freshly shaved, the skin scarified with a knife, and a portion of the spleen of guinea pig number 1766, which had succumbed this date to acute pest infection, was rubbed well over the scarified area. Three control guinea pigs were inoculated in the same manner. The monkeys were inoculated subcutaneously with 0.33 cubic centimeter of a suspension of "Pest Virulent" (5 cubic centimeters of bouillon to one 48-hour agar slant culture from guinea pig number 1764, second generation, equal to about ⅔ oese.

Inoculations in guinea pigs.

Animal No.	Inoculated February 7 with—	Infected February 24 with—	Result.	Autopsy and remarks.
1731	5 cc. artificial aggressin intraperitoneally.	Abdomen massaged with portion of pest spleen.	Dead Mar. 1, after 5 days.	Typical buboes containing numerous pest bacilli.
1732	——do——	——do——	——do——	Do.

SERIES 29—Continued.

CONTROLS.				
Animal No.	Inoculated February 7 with—	Infected February 24 with—	Result.	Autopsy and remarks.
1770		Abdomen massaged with portion of pest spleen.	Dead Feb. 27, after 3 days.	Innumerable pest bacilli, buboes, etc.
1771		do	Dead Mar. 1, after 5 days.	Do.
1772		do	Dead Mar. 5, after 9 days.	Do.

Inoculations in monkeys.

Animal No.	Inoculated February 7 with—	Infected February 25 with—	Result.	Autopsy and remarks.
1733	5 cc. artificial aggressin subcutaneously.	About ⅔ oese "Pest Virulent" subcutaneously.	Dead Mar. 2, after 5 days.	Bubo containing pus and typical pest bacilli.
1734	do	do	Alive and well.	
1735	do	do	Dead Mar. 1, after 4 days.	Very numerous pest bacilli in smears from spleen.
1736	do	do	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from spleen.
1737	do	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1738	do	do	Dead Mar. 1, after 4 days.	Fair numbers of pest bacilli in smears from spleen.
1739	2.5 cc. artificial aggressin subcutaneously.	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1740	do	do	Dead Feb. 28, after 3 days.	Smears from spleen negative for pest bacilli. Smears from point of inoculation show innumerable pest bacilli.
1741	do	do	Dead Mar. 1, after 4 days.	Innumerable pest bacilli in smears from spleen.
1742	do	do	Dead Mar. 2, after 5 days.	Œdema and hæmorrhage about point of inoculation. Numerous pest bacilli.
1743	1 cc. artificial aggressin subcutaneously.	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1744	do	do	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from the spleen.

SERIES 29—Continued.

Animal No.	Inoculated February 7 with—	Infected February 25 with—	Result.	Autopsy and remarks.
1773	5 cc. artificial aggressin subcutaneously.	About $\frac{2}{3}$ oese "Pest Virulent" subcutaneously.	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from the spleen.
1774	do	do	do	Do.
1775	do	do	Dead Feb. 27, after 2 days.	Do.
1776	do	do	Dead Feb. 28, after 3 days.	Do.
1777	do	do	do	Do.
1778	do	do	do	Do.
1779	do	do	Alive and well.	
1780	do	do	Dead Feb. 28, after 3 days.	Do.
1781	do	do	do	Do.
1782	do	do	do	Do.
1783	do	do	Alive and well.	
1784	do	do	do	
1785	do	do	Dead Feb. 28, after 3 days.	Do.
1786	do	do	do	Do.
1787	do	do	Dead Mar. 1, after 4 days.	Do.
1788	do	do	Dead Feb. 28, after 3 days.	Do.
1789	do	do	do	Do.
1790	do	do	Dead Mar. 1, after 4 days.	Do.
1791	do	do	Dead Mar. 3, after 6 days.	Do.
1792	do	do	Dead Mar. 1, after 4 days.	Do.
		CONTROLS.		
1793		About two-third oese "Pest Virulent" subcutaneously.	Dead Feb. 27, after 2 days.	Do.
1794		do	do	Do.
1795		do	Dead Feb. 28, after 3 days.	Do.
1796		do	do	Do.
1797		do	do	Do.

SERIES 30.—*Inoculations with artificial pest aggressin in guinea pigs.*

February 11: The animals of this series were inoculated with Artificial Aggressin I, details of the preparation of which is given in Series 29. The aggressin tested just before inoculation proved to be sterile. The animals were reinoculated on February 26 by the method described for the guinea pigs in Series 29. (See p. 223.)

Animal No.	How inoculated.	Reinoculated February 26.	Result.	Autopsy and remarks.
1753	4.5 cc. Artificial Aggressin I intraperitoneally Feb. 11.	Abdomen shaved and scarified and massaged with portion of spleen of guinea pig No. 1768 which died this day of plague.	Dead Mar. 1, after 13 days.	Typical pest buboes.
1754	do	do	do	Hæmorrhagic buboes. Numerous pest bacilli in smears.
		CONTROLS.		
1798		Abdomen shaved and scarified and massaged with portion of spleen of guinea pig No. 1768 which died this day of plague.	Dead Mar. 1, after 3 days.	Pest. Typical buboes.
1799		do	do	Do.
1800		do	Dead Mar. 2, after 4 days.	Pest bacilli in smears from spleen.

SERIES 31.—*Inoculations with artificial pest aggressin in guinea pigs.*

May 11: Thirty-six 48-hour, large, agar slant cultures * (from second generation, guinea pig number 1956) were suspended in 50 cubic centimeters of distilled water. The suspension was then placed on an electrical shaking machine and was thoroughly shaken for three days. On May 14 it was removed and cultures taken which later developed a pure growth of pest bacilli. Five per cent carbolic acid was then added in sufficient quantity to form a 0.5 per cent solution. The suspension was then centrifuged for five hours at a velocity of 4,000 revolutions per minute and the clear fluid above, pipetted from the bacterial sediment. Cultures from this fluid remained sterile. The animals were inoculated as below. On June 11, one month after the first injection, the immunity of the animals was tested in the following manner: One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 2067, second transplant) was suspended in 5 cubic centimeters of bouillon, and 5 oesen of this suspension then rubbed over

* The culture tubes designated in these experiments as "large" contain a surface of agar of more than twice the area of that in the tubes ordinarily employed in bacteriological work.

SERIES 31—Continued.

a shaved area on the abdomen and the skin lightly scarified with a scalpel. Five control animals were all inoculated in the same manner.

Animal No.	Inoculated May 14 with—	Reinoculated June 11 with—	Result.	Autopsy and remarks
1969	5 cc. Artificial Aggressin II intraperitoneally.	5 oesen suspension of "Pest Virulent."	Dead June 16, after 5 days.	Innumerable pest bacilli in smears from spleen.
1970	do	do	Dead June 18, after 7 days.	Numerous pest and post-mortem invading bacilli in smears from spleen.
1971	do	do	do	Numerous pest bacilli in smears from spleen.
1972	do	do	do	Do.
1973	do	do	Alive and well.	
1974	do	do	Dead June 21, after 9 days.	Pest bubo. Numerous pest bacilli in smears from spleen.
		CONTROLS.		
2093		5 oesen suspension of "Pest Virulent."	Dead June 16, after 5 days.	Pest.
2094		do	Dead June 18, after 7 days.	Numerous pest bacilli in smears from spleen. Hæmorrhagic buboes.
2095		do	Dead June 16, after 5 days.	Pest.
2096		do	do	Do.
2097		do	Dead June 18, after 7 days.	Numerous pest bacilli in smears from spleen.

SERIES 36.—*Inoculations with artificial pest aggressin in guinea pigs.*

May 16: Sixty-seven 48-hour large agar slant cultures of "Pest Virulent" (from guinea pig number 1958, second generation) were suspended in 67 cubic centimeters distilled water. The suspension was then placed in a bottle, afterwards sealed and throughly shaken on an electrical machine for three days. On May 19 cultures were taken, these later showed numerous pest bacilli still alive. A 5 per cent solution of carbolic acid was added to the suspension sufficient to form a 0.5 per cent solution and it was then centrifuged for three hours at a velocity of 4,000 revolutions per minute. The centrifugated tubes were then removed and placed for two hours at a temperature between 45° and 46° C. The clear fluid above was then pipetted off and, after its sterility was proved, used on May 20 for the inoculations described in the table below. On July 16, two months later, the animals were all reinoculated in the following manner: 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2174, second transplant) in 5 cubic centimeters of bouillon were rubbed over a shaved area on the abdomen which was scarified with a scalpel.

SERIES 36—Continued.

It will be seen that these animals were inoculated in exactly the same manner as those used in the experiments described in Series 32 and 33. (See pp. 204, and 218.) The same twenty-five control guinea pigs, numbered 2098 to 2122, inoculated in the same manner, served for the present series as well. All of them died of acute pest infection.

Animal No.	Immunized May 20, with—	Infected July 16, with—	Result.	Autopsy and remarks.
2018	5 cc. Artificial Aggressin III.	5 oesen suspension "Pest Virulent" massaged over abdomen.	Dead July 22, after 6 days.	Moderate number of pest bacilli in smears from spleen.
2019	do	do	Dead July 23, after 7 days.	Innumerable pest bacilli in smears from spleen.
2020	do	do	do	Typical pest spleen.
2021	do	do	Dead July 21, after 5 days.	Few pest bacilli in smears from spleen.
2022	do	do	Dead July 23, after 7 days.	Typical pest spleen.
2023	do	do	Dead July 25, after 9 days.	Advanced bubo. Innumerable pest bacilli in smears from spleen.
2024	do	do	Dead July 23, after 7 days.	Numerous pest bacilli in smears from spleen.
2025	do	do	do	Few pest bacilli in smears from spleen.
2026	do	do	Dead July 25, after 9 days.	Typical pest spleen.
2027	3 cc. Artificial Aggressin III.	do	Dead July 23, after 7 days.	Innumerable pest bacilli in smears from spleen.
2028	2 cc. Artificial Aggressin III.	do	Dead July 25, after 8 days.	Numerous pest bacilli in smears from spleen.
2029	2½ cc. Artificial Aggressin III.	do	Dead July 23, after 7 days.	Do.

SERIES 42.—*Inoculations with artificial pest aggressin in guinea pigs.*

On July 16, sixty 48-hour agar cultures of the strain "Pest Virulent" (from guinea pigs numbered 2174 and 2175, second transplant) were suspended in 60 cubic centimeters distilled water, 1 cubic centimeter to each culture. The suspension was then placed on an electrical shaking machine for five days. The preparation of the artificial aggressin from this point on was then continued in exactly the same manner as described in Series 29 (p. 223). After it was centrifuged, cultures from the clear fluid showed the aggressin to be sterile.

On July 23 each guinea pig was inoculated subcutaneously with 5 cubic centimeters and again on August 1, each animal receiving a repetition of this dose. On August 24, about three weeks after the second inoculation the immunity of the animals was tested by massaging the abdomen with a portion of the spleens of guinea pigs numbered 2315 and 2316, just dead of acute plague infection. Two control animals were inoculated at the same time and in the same manner.

SERIES 42—Continued.

Animal No.	Immunized July 23 with—	Second immunization August 1.	Infected August 24.	Result.	Autopsy and remarks.
2197	5 cc. Artificial Aggressin IV.	5 cc. Artificial Aggressin IV.	Abdomen massaged with portions of the spleens of guinea pigs numbered 2315 and 2316 just dead of acute plague infection.	Dead Aug. 29, after 6 days.	Numerous pest bacilli in smears from spleen.
2198	do	do	do	Dead Sept. 2, after 9 days.	Subacute pest.
2199	do	do	do	Alive and well	
2200	do	do	do	do	
			CONTROLS.		
2322			Abdomen massaged with portions of the spleens of guinea pigs numbered 2315 and 2316 just dead of acute plague infection.	Dead Aug. 29, after 5 days.	Pest bacilli in smears from spleen.
2323			do	Dead Aug. 31, after 7 days.	Do.

SERIES 34.—*Animals inoculated for the preparation of natural plague aggressin.*

Animal No.	Inoculated intraperitoneally with—	Result.	Abdominal exudate collected.
Rabbit 1966*	2 oesen of the heart's blood of guinea pig 1958 in 10 cc. bouillon on May 14.	Dead May 20, after 6 days.	2.75 cc. for Aggressin E.
Guinea pig 1964.	Portion of spleen of guinea pig No. 1957 shaken up in 5 cc. bouillon May 13.	Dead May 14, after 1½ days.	5 cc. for Aggressin A.
Guinea pig 1999.	½ oese "Pest Virulent" 3 days' culture, first transplant from guinea pig No. 1957 in 10 cc. bouillon May 17.	Dead May 19, after 2 days.	4 cc. for Aggressin C.
Guinea pig 2000.	do	Dead May 18, after 1 day.	4 cc. for Aggressin B.
Guinea pig 2001.	do	Dead May 19, after 2 days.	2 cc. for Aggressin C.
Guinea pig 2002.	do	Dead May 18, after 1 day.	4.5 cc. for Aggressin B.
Guinea pig 2003.	do	do	4 cc. for Aggressin B.
Guinea pig 2004.	do	do	3 cc. for Aggressin B.
Guinea pig 2007.	½ oese 6 days' culture "Pest Virulent" from guinea pig No. 1958 first transplant in 10 cc. bouillon.	Dead May 19, after 1 day.	3 cc. for Aggressin C.
Guinea pig 2008.	do	do	1.75 cc. for Aggressin D.
Guinea pig 2009.	do	Dead May 20, after 2 days.	4.5 cc. for Aggressin E.
Guinea pig 2010.	do	Dead May 19, after 1 day.	4.5 cc. for Aggressin D.
Guinea pig 2011.	do	Dead May 20, after 2 days.	2 cc., foul, containing many post-mortem bacilli. Not used.

*A number of rabbits were inoculated for the purpose of preparing natural aggressin. However, these animals were found in Manila to be unsatisfactory for this purpose owing to the rapid contamination of the fluid in the pleural or abdominal cavity due to post-mortem decomposition.

SERIES 34—Continued.

The abdominal exudates from these guinea pigs were collected on the day of the death of the animal and mixed with an equal volume of normal saline solution. Five per cent carbolic acid was then added in sufficient amount to form a 0.5 per cent solution. The mixtures were then heated for two hours at 44° to 45° C. and subsequently centrifuged for four hours at a velocity of 4,000 revolutions per minute. The clear fluid above was then pipetted and used in the inoculations described in Series 35. Its sterility was in each instance demonstrated.

SERIES 43.—*Guinea pigs inoculated for the preparation of natural plague aggressin.*

Animal No.	Inoculated intraperitoneally with—	Result.	Exudate obtained.
2201	1 oese "Pest Virulent" from guinea pig No. 2179 first transplant in 10 cc. bouillon July 23.	Dead July 26, after 3 days	5 cc. for Aggressin F.
2202	do	Dead July 25, after 2 days	3.5 cc. foul exudate obtained. Not used.
2203	do	Dead July 28, after 5 days	5 cc. for Aggressin G.
2210	½ oese 6-day culture of "Pest Virulent" guinea pig No. 2183 first transplant, in 5 cc. bouillon July 30.	Dead July 31, after 1 day	1 cc. for Aggressin H.
2211	do	Dead Aug. 1, after 2 days	3 cc. for Aggressin I.
2212	do	Dead July 31, after 1 day	1 cc. for Aggressin H.
2213	do	do	2 cc. for Aggressin H.
2214	do	do	1 cc. for Aggressin H.
2215	do	Dead Aug. 1, after 2 days	1 cc. for Aggressin I.
2216	do	Dead July 31, after 1 day	4 cc. for Aggressin H.
2217	do	do	Do.
2218	do	do	Do.
2219	do	Dead Aug. 1, after 2 days	2.5 cc. for Aggressin I.

The abdominal exudates from these guinea pigs were collected on the day of the death of the animal and mixed with an equal volume of normal saline solution. Five per cent carbolic acid was then added, sufficient to form a 0.5 per cent solution. The mixture was then heated for from one to two hours at a temperature of 45° to 46° C. and subsequently centrifuged for four to five hours at a velocity of 4,000 revolutions per minute. The clear fluid above was then pipetted off and used in the inoculations described in Series 44. The sterility of the aggressin was demonstrated in each instance.

SERIES 35.—*Inoculations with natural plague aggressin in guinea pigs.*

The animals were inoculated as below with natural aggressin, the preparation of which is described in Series 34. On July 16 they were all, with the exception of numbers 2053 and 2054 reinoculated by rubbing 5 oesen of a suspension of "Pest Virulent" (5 cubic centimeters bouillon to one 48-hour culture guinea pig numbered 2174, second transplant) over a shaved and lightly scarified area on the abdomen. These animals were inoculated in exactly the same manner as those given in Series 32 and 33 (see pp. 204 and 218), and the same twenty-five control guinea pigs numbered 2098 to 2122 were employed for the present series. Guinea pigs numbered 2053 and 2054 were inoculated June 18 by massaging a shaved area over the abdomen with a portion of the spleen of guinea pig numbered 2124 and scarifying the skin with a scalpel. Two control guinea pigs, numbered 2140 and 2141, were also inoculated in the same manner.

SERIES 35—Continued.

Animal No.	Immunized intraperitoneally with—	Reinoculated with—	Result.	Autopsy and remarks.
2012	5 cc. Natural Aggressin B, May 19.	5 oesen suspension of "Pest Virulent" over abdomen July 16.	Dead July 26, after 10 days.	Pest bubo. Innumerable pest bacilli in smears from spleen.
2013	do	do	Dead July 25, after 9 days.	Pest bubo. Very numerous pest bacilli in smears from spleen.
2014	4 cc. Natural Aggressin B, May 19.	do	Alive	
2015	do	do	do	
2016	2 cc. Natural Aggressin B, May 19.	do	do	
2017	do	do	do	
2030	3 cc. Natural Aggressin C, May 20.	do	Dead July 23, after 7 days.	Numerous pest bacilli in smears from spleen.
2031	do	do	do	Do.
2032	do	do	do	Pest. Typical bubo and spleen.
2033	3.5 cc. Natural Aggressin C, May 20.	do	Dead July 25, after 9 days.	Typical pest spleen. Fair number of pest bacilli in smears from spleen.
2034	2.5 cc. Natural Aggressin D, May 20.	do	Dead July 24, after 8 days.	Pest. Numerous pest bacilli in smears from spleen.
2035	do	do	Dead July 31, after 15 days.	Innumerable pest bacilli in smears from spleen.
2006	5 cc. Natural Aggressin A, May 15.	do	Dead July 26, after 6 days.	Numerous pest bacilli in smears from spleen.
2053	4.5 cc. Natural Aggressin E, May 21.	Abdomen massaged with portion of spleen of guinea pig No. 2124 on June 18.	Dead June 22, after 4 days.	Innumerable pest bacilli in smears from spleen.
2054	do	do	Dead June 25, after 7 days.	Comparatively few bacilli in smears from spleen.
		CONTROLS.		
2140		Abdomen massaged with portion of spleen of guinea pig No. 2124 on June 18.	Dead June 25, after 7 days.	Innumerable pest bacilli in smears from spleen.
2141		do	Dead June 27, after 9 days.	Do.

And twenty-five other controls; all dead. (See Series 32 and 33, pp. 204 and 218.)

SERIES 44.—*Inoculations with natural plague aggressin in guinea pigs.*

The animals were inoculated intraperitoneally July 27 to August 3 as given below with natural aggressin, the preparation of which is described in Series 43 (p. 230). On August 28, about one month after the first inoculation, they were all reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour slant culture from guinea pig numbered 2315 second transplant in 10 cubic centimeters bouillon) being rubbed over a freshly shaved area of the abdomen of the animal and the skin well scarified with a scalpel. Twenty-five control animals, numbered 2362 to 2386, were also inoculated in exactly the same manner and all died. The details of these autopsies were given in Series 41 (p. 210).

Animal No.	Inoculated intraperitoneally with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2205	July 27, 4.5 cc. Natural Aggressin F.	5 oesen suspension "Pest Virulent" rubbed on abdomen.	Dead Sept. 4, after 7 days.	Numerous pest bacilli in smears from spleen.
2206	do	do	Alive and well.	
2225	July 30, 4.5 cc. Natural Aggressin G.	do	Dead Sept. 15, after 18 days.	Typical bubo. No organisms found in smears from spleen.
2226	do	do	Alive and well.	
2232	Aug. 1, 4 cc. Natural Aggressin H.	do	do.	
2233	do	do	Dead Sept. 5, after 8 days.	Fair numbers of pest bacilli in smears from spleen.
2234	do	do	Dead Sept. 8, after 11 days.	Pest bubo. One or two abscesses in spleen.
2235	do	do	Dead Sept. 4, after 7 days.	Numerous pest bacilli in smears from spleen.
2236	do	do	Dead Sept. 7, after 10 days.	Innumerable pest bacilli in smears from spleen.
2237	do	do	Dead Sept. 4, after 7 days.	Do.
2269	Aug. 3, 4 cc. Natural Aggressin I.	do	Alive and well.	
2270	do	do	Dead Sept. 9, after 12 days.	Innumerable pest bacilli in smears from spleen.

SERIES 54.—*Inoculations with Klein's method in guinea pigs.*

The buboes, lungs, spleen and liver containing necrotic foci were removed from three guinea pigs which had died of subacute plague infection. The organs were finely minced, spread out on glass in thin layers over sulphuric acid and dried at from 46° to 47° C. for four days, and at 45° C. for two days longer. The dried material was then rubbed up in a mortar with saline solution, the fluid portion being decanted off from the sediment which did not dissolve.

Each animal of the series received subcutaneously 2 cubic centimeters of this suspension, equal to about 25 milligrams of the powder. In two of the animals, numbered 2822 and 2823, large sloughs of the skin followed the inoculation. On November 26, about one month after the injection, the immunity of the animals was tested with the strain "Pest Virulent" in exactly the same manner as was

SERIES 54—Continued.

that of the animals comprising Series 46, 47, and 50 (pp. 211, 220 and 197). Twenty-five control animals, numbers 2870 to 2894, were also inoculated at the same time and in the same manner. All succumbed from acute plague infection. For the details of the protocols see Series 46.

Animal No.	Inoculated October subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2821	2 cc. suspension of dried plague organs.	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical bubo. Pest bacilli in spleen.
2822	do	do	do	Do.
2823	do	do	Dead Dec. 5, after 9 days	Do.
2824	do	do	Alive and well	
2825	do	do	do	
2826	do	do	Dead Dec. 3, after 7 days	Do.

SERIES 56.—*Inoculations by Klein's method in guinea pigs.*

The buboes, lungs, spleen and liver containing necrotic foci were removed from three guinea pigs, numbered 3017, 3018, and 3025 which had died of plague infection and were used for the manufacture of the prophylactic employed in these experiments. The prophylactic was prepared after the method of Klein as described in Series 54 (p. 232). Each animal received 2 cubic centimeters of the suspension. In three of the animals sloughs of the skin followed the inoculations. About one month after the inoculations the immunity of the animals was tested by rubbing 5 oesen of a suspension of "Pest Virulent" (one 48-hour agar slant culture from guinea pig number 3100, second transplant in 5 cubic centimeters bouillon) over a shaved area on the abdomen. Three control animals inoculated in the same manner and at the same time all succumbed to pest infection.

Animal No.	Inoculated subcutaneously with—	Reinoculated March 12 with—	Results.	Autopsy and remarks.
3035	2 cc. suspension dried plague aggressin.	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Mar. 16, after 4 days	Pest.
3036	do	do	Dead Mar. 20, after 8 days	Do.
3037	do	do	Alive and well	
3038	do	do	Dead Mar. 17, after 5 days	Pest.
3040	do	do	Dead Mar. 16, after 4 days	Do.
3046	do	do	Alive and well	
3047	do	do	Dead Mar. 18, after 6 days	Pest.
		CONTROLS.		
3052		5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Mar. 17, after 5 days	Pest.
3055		do	Dead Mar. 16, after 4 days	Do.
3056		do	Dead Mar. 16, after 4 days	Do.

DURATION OF IMMUNITY AFTER VACCINATION.

SERIES 55.—*Demonstrating duration of immunity in monkeys after vaccination.*

The animals comprising this series were vaccinated with the strains "Pest Avirulent" and "Maassen Alt" at different times, as may be seen from the table below. From nine to ten months after the vaccination they were all collected and on November 12 reinfected by the inoculation of $\frac{2}{3}$ oese of the strain "Pest Virulent" (from guinea pig 2764, second transplant).

Animal No.	Vaccinated.	Reinoculated November 12.	Result.	Autopsy and remarks.
2842	Jan. 10	$\frac{2}{3}$ oese "Pest Virulent."	Dead Nov. 19, after 7 days	Pest bubo containing few organisms. No bacilli in smears from spleen.
2843	Jan. 12	do	Alive and well	
2844	Jan. 15	do	Dead Nov. 16, after 4 days	Numerous pest bacilli in smears from spleen.
2845	Jan. 16	do	Alive and well	
2846	Jan. 18	do	Dead Nov. 20, after 8 days	Few swollen pest bacilli in smears from spleen.
2847	Jan. 22	do	Dead Dec. 1, after 19 days	Chronic bubo containing few pest bacilli.
2848	Jan. 25	do	Dead Nov. 17, after 5 days	Numerous pest bacilli in smears from spleen.
2849	Jan. 27	do	Alive and well	
2850	Jan. 29	do	do	

VII. SUMMARY OF THE RESULTS OF THE ANIMAL EXPERIMENTS IN IMMUNIZATION BY THE DIFFERENT METHODS.

It will be seen from a study of the different series of experiments described on pages 187 to 234 that in many instances the results in immunization obtained by the same method of inoculation show considerable variation. This is particularly so in the series of monkeys inoculated in the early part of the work where the immunity of the animals in the different series was tested with varying amounts of the virulent pest organism. However, many difficulties were also encountered in the series in which the experiments were performed later. In the beginning of this article and in the records of some of the experiments, attention was called to the fact that the lethal dose for the species of monkeys employed varied considerably. In some instances over fifty times the lethal dose for one animal will not cause the death of another of about the same size. For this reason and because occasionally, in the earlier experiments, a control animal did not succumb to pest infection, a very large multiple of the lethal dose for the average monkey was finally employed; therefore, the percentage of these animals immunized was not as high as it might otherwise have been. It is undoubtedly for this reason that so low a percentage of the monkeys which had previously been inoculated with killed cultures of the pest organism subsequently resisted the infection. On the other hand, in my experiments with guinea pigs inoculated with killed cultures, the percentage of immunity obtained was as high as or higher than that frequently encountered by other authors.

Owing to this great individual variation in the susceptibility of different monkeys to pest infection, a fact discovered during the course of the inoculations, these animals did not prove to be as suitable as guinea pigs for the comparison of the value of the different methods. However, on the other hand, as has been mentioned above, the behavior of the monkey in relation to its resistance and immunity to pest infection probably much more closely resembles that encountered in man.

It is obvious that the series of inoculations which have been described are not selected as ideal ones. The entire number performed in relation to immunization by the different methods has been recorded in the order in which the experiments were carried on, the unfavorable as

well as the favorable results being detailed. By no method was it possible by the employment of a single inoculation and a fixed dose, thoroughly to protect all of the animals of a series against infection. In fact a single vaccination of a number of animals of the series, even though the dose was so large as to cause the death of some of them, would not usually render thoroughly immune more than 75 to 80 per cent of the remainder. (See experiments with the strain "Maassen Alt.")

In spite of variations in the results of immunization sometimes obtained in the different series by the same method of inoculation, nevertheless, an examination of the experiments demonstrates conclusively and beyond any doubt the great value of vaccination against plague infection and its evident superiority to the other methods of immunization. No doubt of this fact is left after a study of the combined table of inoculations on page 238 where a comparison of the value of the different methods may be made at a glance.

In relation to the time which the immunity takes to develop and the time which it persists after vaccination, it may be stated that in three instances attempts were made to infect monkeys numbered 1581, 1593 and 1626 which had been vaccinated only six days previously. All of these animals remained alive and well. Nine monkeys, numbered 2842 to 2850 (see Series 55, p. 234), which had been vaccinated with the strain "Pest Avirulent" or "Maassen Alt" between nine and ten months previously were collected and their immunity tested by the inoculation of $\frac{2}{3}$ oese of the strain "Pest Virulent;" five of the animals succumbed and four (44 per cent) remained alive. In connection with these experiments it must be emphasized that the animals during the entire time between the vaccination and the infection (nine to ten months) had been in close captivity and were not in particularly good physical condition at the time they were infected. Moreover, it is obvious that in testing their immunity a much greater amount of plague bacilli ($\frac{2}{3}$ oese of the virulent strain) was employed than either man or animals would ever receive from a natural plague infection.

Prophylactic inoculations of natural aggressin appear to be next in value to *vaccination* as a means of immunization against pest. Inoculations with artificial plague aggressin did not, in my experiments, prove to be nearly as efficacious as those with natural aggressin. A much higher immunity was obtained with the latter prophylactic. However, as I have already pointed out elsewhere,[49] there was apparently no difference in the quality of the immunity obtained with the natural

[49] *This Journal* (1906), 1, 512.

aggressin from that produced by the artificial product, and my subsequent experiments, as did my earlier ones, have only further confirmed the views of Wassermann and Citron [50] that the aggressins must be considered to be hypothetical substances and that, so far as their immunizing value is concerned, in these exudates we have to do mainly with the sustances extracted from the bacilli themselves. Evidently, in the case of the plague bacillus, the receptors of the organism in the so-called aggressin exudates of animals become liberated in a more efficient manner for immunization and probably exist in a less altered condition than they do in the aqueous suspension of the bacilli obtained by artificial means. Obviously, in natural plague aggressin no other immunizing substances are existent than those present in the prophylactic against pest recommended by Terni and Bandi. The two methods are practically identical as Bandi [51] has recently pointed out. However, it must be admitted that Bandi did not originally explain the principle of the action of his prophylactic, as we understand its action to-day after a study of the subjects of free receptors and of aggressins. The method of inoculation with natural plague aggressin is not likely to come into general use because of the great difficulties encountered in the preparation of the prophylactic. Moreover, in my experiments I have not obtained the satisfactory results with it which Hueppe and Kikuchi evidently anticipated. The method of *vaccination,* as already mentioned, gives a much greater degree of protection. Although Klein's method, according to the small number of experiments I have performed, gives about the same results in immunizing guinea pigs as are obtained by the inoculations with natural aggressin, yet the injection of the former substance as prepared in my experiments produced a much greater local reaction than the latter. Therefore, I could not ascertain that this method had any particular advantage over that in which inoculations of natural aggressin were employed.

I performed no experiments with Lustig's plague prophylactic, partly because of the poor results which Kolle and Otto [52] encountered in their experiments and partly because the results which have been obtained in this laboratory by the use of this method in extracting the immunizing substances from the cholera spirillum have not been encouraging for its further use; other methods of extraction of these substances from the bacilli having in my opinion given more satisfactory results.

[50] *Deutsche Med. Wchnschr.* (1905), **31**, 1101.
[51] *Centrbl. f. Bakteriol.* (1906), **42**, 448.
[52] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 517.

Combined table comparing efficiency of different methods of immunization.

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized. Number.	Immunized. Percentage.
KILLED PEST CULTURES.				
Monkeys (bouillon cultures):				
Series 5	8		3	37
Series 49	9		2	22
Monkeys (agar cultures):				
Series 9	3			
Series 23*	[18]	[3]	[11]	[72]
Series 25	20	3	4	23
Series 48	15	1	4	28
Total	*55	4	13	25
Guinea pigs (killed agar cultures):				
Series 50 (total)	15		4	26
"PEST AVIRULENT."				
Monkeys:				
Series 4	6		5	83
Series 11	5		1	20
Series 12	8		4	50
Series 18	10		5	50
Series 51	15		8	53
Total	44		23	52
Guinea pigs:				
Series 32	11	5	5	83
Series 37	9	2	6	85
Series 39	15		8	53
Series 41	21		15	71
Series 46	15		12	80
Total	71	7	46	72
"MAASSEN ALT."				
Monkeys:				
Series 17	4		3	75
Series 21	12		8	66⅔
Series 24	18	4	13	92
Series 52	15	1	7	50
Total	49	5	31	70

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized. Number.	Immunized. Percentage.
"MAASSEN ALT"—Continued.				
Guinea pigs:				
Series 33	10	3	7	100
Series 38	7	1	4	66⅔
Series 40	15	1	11	78
Series 47	15	1	14	100
Total	47	6	36	88
EXTRACTS OF PLAGUE BACILLUS (FREE RECEPTORS).				
Monkeys:				
Series 7	4		1	25
Series 26	5		1	20
Total	9		2	22
ARTIFICIAL AGGRESSIN.				
Monkeys:				
Series 29 (total)	32		4	12½
Guinea pigs:				
Series 29	2		0	0
Series 30	2		0	0
Series 31	6		1	16⅔
Series 36	12		0	0
Series 42	4		2	50
Total	26		3	11
NATURAL AGGRESSIN.				
Guinea pigs:				
Series 35	15		4	26
Series 44	12		4	33⅓
Total	27		8	30
KLEIN'S METHOD.				
Guinea pigs:				
Series 54	6		2	33⅓
Series 56	7		2	28
Total	13		4	30

*Series 23 has not been considered in the total. See text.

VIII. THE FORMATION OF AGGLUTININS IN PLAGUE IMMUNE SERUM.

After having concluded from the experiments recited above that true plague vaccination (inoculation with living, attenuated cultures) produced the highest immunity, inoculations were made in human beings with the strain "Pest Avirulent." In order to ascertain if any evidences of immunity could be demonstrated in the inoculated, their blood was tested for the presence of agglutinins, anti-infectious substances and opsonins. This led me to investigate in detail whether, and if so, to what extent, these same anti-bodies existed in the blood sera of animals which had been immunized against plague infection.

AGGLUTINATION OF THE PEST BACILLUS.

Wyssokowitz and Zabolotny[53] and the German Plague Commission called attention to the fact that the blood serum of persons who had suffered an attack of plague sometimes showed a weak agglutinating action against the pest bacillus. The German Plague Commission further emphasized that under these circumstances the reaction was not always present and that its occurrence bore no relation to the severity of the disease, since in the most severe case which they examined it was absent, while in a mild case of plague, the strongest positive reaction was obtained. They studied the sera of two persons who about three weeks before had been vaccinated against pest by Haffkine, but found no trace of an agglutinating reaction. They also examined four different pest immune sera produced in horses and in three found practically no agglutinative action or only traces of it; but one of the sera showed a suggestion of a reaction above the dilution of 1 to 20. A goat which received four injections of killed cultures of the pest bacillus, six cultures being finally injected, was killed about one week after the final inoculation. Its blood serum then showed a strong agglutinative action in a dilution of 1 to 30. The commission conclude that in plague infection the agglutination is not at all parallel with the protective and immunizing power of the serum. Pest sera which showed a strong agglutinating reaction, in the animal body proved to be entirely inactive against pest infection and *vice versa*.

Zabolotny[54] also found that the agglutinative action of the blood serum of those who had suffered with pest was very inconstant. In some cases which had been inoculated with Haffkine's prophylactic, he found a weak agglutinative power which, however, was not so marked as in cases which had recovered from a natural attack of the disease.

Vagedes[55] investigated the agglutinative action of the sera of thirteen persons who had suffered with pest in Oporto. In only two cases was a very weak,

[53] *Ann. d. Pinst. Pasteur* (1897), **11**, 662.

[54] *Arch. d. Sci. Biologiques* (1901), **8**, 85.

[55] *Klin. Jahrbch.* (1900), **7**, 537, and *Arb. a. d. k. Gsndhtsamte Berl.* (1900), **17**, 181.

positive result obtained. The blood serum was collected from one to four months after the beginning of the illness.

Uriate [56] in a study of epidemics of pest in Paraguay, Rosario and Buenos Ayres, found that the sero-agglutination of the pest organism occurred in cases of plague only late in the course of the disease and then very irregularly. It failed actually in more than 300 cases which, from a bacteriological and clinical standpoint, were certainly those of pest.

On the other hand Paltauf,[57] Markl [58] and Kolle and Martini [59] showed that a specific agglutination of the organism occurred in various dilutions with pest immune sera produced in horses and other animals by repeated injections of the plague bacillus. Paltauf found that in horses immunized by repeated inoculations a reaction occurred in dilutions of 1 to 20. A distinct agglutination occurred within one hour in dilutions of 1 to 100 with the sera with which Markl worked. Kolle and Martini with less virulent cultures of the pest bacillus obtained a reaction within one hour in dilutions of 1 to 1,000 to 1 to 6,000. Kolle and Otto [60] in the study of two pest immune sera found that agglutination occurred within fifteen minutes in as high dilutions as 1 to 400. Klein [61] also reported that the blood of vaccinated guinea pigs showed an agglutinative value in dilutions of 1 to 20 or 1 to 30, the reaction occurring in fifteen minutes.

The German Plague Commission and Kossel and Overbeck [62] emphasized the difficulty or impossibility of securing a suspension of the freshly grown pest bacillus which microscopically was free from clumps of the organism, and therefore they recommended that the test should be made macroscopically in the test tube and the reaction observed with the assistance of a hand lens. The time recommended for the observation of the test was from one-half to one hour, the suspension being placed in the incubator at 37° C. for this period. However, Kolle and Martini recommended that the reaction be noted after five minutes, since in their experience spontaneous precipitation of the bacteria sometimes occurred after one hour.

Klein [63] pointed out that cultures of the pest bacillus on agar possess a sticky, viscid growth due to the production of a gelatinous interstitial substance which is insoluble in bouillon. He therefore recommended physiological salt solution as a medium for preparing the suspension. He also found that the addition of bouillon to a saline suspension of the pest organism caused clumping of the bacteria.

Gauthier and Raybaud [64] found that precipitation of the bacteria also frequently occurred with plague cultures grown on gelatine and suspended in saline solution and that after two hours a spontaneous, flocculent sedimentation which resembled true agglutination was apt to form.

In my studies of the agglutination of the pest bacillus, I have frequently found the same difficulty in securing complete suspensions of the organisms owing to their sticky, viscid growth, even when saline

[56] Mitt. a. d. sektion f. Hyg. d. Kongress des Ass. francaise pour l'avancement des sci. tenu Grenoble 1904, *Arch. f. Schiffs u. Trop. Hyg.* (1905), **9**, 89.

[57] *Wien. Klin. Wchnsch.* (1897), **10**, 537.

[58] *Centrbl. f. Bakteriol.* (1901), **29**, 810.

[59] *Deutsche med. Wchnsch.* (1902), **28**, 46.

[60] *Ztschr. f. Hyg. u. Infectionskrankh.* Leipz. (1902), **40**, 595.

[61] *Arb. a. d. k. Gsndhtsamte,* Berl. (1902), **18**, 114.

[62] *Lancet* (1901), **1**, 456, 1535.

[63] *Ibid.*

[64] *Compt. rend. Soc. de biol.*, Par. (1904), **56**, 391.

solution has been used. The method employed in making these suspensions has been as follows:

One cubic centimeter of an 0.85 per cent saline solution has been placed in a small test tube and 1 oese of a 24-or 48-hour agar culture of the organism has been introduced into the tube, thoroughly rubbed up against the walls by means of the oese and the bacteria gradually moistened with the saline solution. While the suspensions obtained with some pest cultures in this manner are satisfactory, a complete suspension of the organism can not be produced with others and it is necessary to combine the suspensions and either filter or allow them to stand until the larger clumps of bacteria have settled to the bottom of the tube, when the supernatant fluid may be drawn off with a pipette.

However, while satisfactory suspensions are apparently obtained by these methods, the greatest difficulty is experienced with certain cultures of this organism in keeping the bacteria in suspension for a longer period than, at most, one or two hours. After this time the organisms begin to precipitate and sometimes at the end of two or three hours the bacteria may have settled almost entirely to the bottom of the tube, the supernatant fluid above appearing almost clear. This phenomenon is particularly marked with the much attenuated plague cultures.

I have followed the suggestion of Shibayama [65] of growing the bacillus in the ice box at a temperature of from 5° to 8° C., with the object of obtaining a less sticky and viscid growth of the pest bacillus and thus securing a more satisfactory suspension of the organism in saline solution. However, while I have found that with cultures which have grown for a number of generations at such a temperature a much less viscid growth apparently is obtained and one which may easily be suspended in the saline solution, on the other hand the bacteria so grown become spontaneously precipitated from the saline suspensions in a much shorter time than do the organisms of the same strain grown at a temperature of 30° C. I have also found that the addition of normal serum to suspensions of the pest bacilli in saline solution sometimes retards and sometimes increases the tendency of the bacteria to settle out of the fluid. For these reasons pseudo-reactions are not infrequently encountered in performing agglutination tests with the plague bacillus, and great care is sometimes necessary to distinguish such reactions from those of true agglutination. Therefore, it is advisable to perform in duplicate all tests for agglutination with the different dilutions of the serum and at the same time to carry on a parallel series of experiments in the same dilutions with a normal serum from an animal of the same species and, moreover, to repeat all the tests with another transplant of the same culture upon the following day. Only by taking these precautions is it possible to distinguish certain pseudo-agglutinations of the pest bacillus from true ones. When testing the

[65] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 482.

agglutinative reaction of supposed plague immune sera, I have occasionally encountered pseudo-agglutinative reactions in the control tubes containing normal serum and suspensions of plague bacilli, which could not be distinguished from those of true agglutination. In the tubes in some instances were seen typical, flocculent precipitates, visible to the naked eye and which settled to the bottom, leaving the overlying liquid clear. Such reactions occurred at the same time in the same dilutions of the immune serum which was being tested, but not in the test tubes containing the suspensions of bacilli alone, and hence if the control tubes with normal serum had not been prepared, the reactions unquestionably would have been considered as true agglutinative ones, which they certainly were not. It is true that the precipitation obtained in these instances disappeared to a greater or less extent on shaking the tube and it then re-formed slowly, but notwithstanding statements in the literature to the contrary, the same phenomenon (disappearance of the precipitation on shaking) occurs in the true agglutination not only of plague but also of cholera, and certain other microorganisms.

From what has been said above it is evident that the time limit for the completion of the agglutinative reaction of the plague bacillus is very important. Most observers have limited the time of the reaction to within two hours. However, Shibayama,[66] who concluded that 72-hour cultures of the plague bacillus, cultivated in the ice box, are agglutinatined in much higher dilutions than are the same strains grown at 32° to 37° C., drew his conclusions from an examination of the reaction after twenty-four hours. In this connection I here shall record some recent experiments performed with two sera obtained from monkeys which had been immunized against plague and with one serum from, a normal monkey. The agglutinative values of these three sera were tested in dilutions of 1 to 10 and 1 to 20, first with 72-hour cultures of a virulent pest strain which had been cultivated for six generations in the ice box at a temperature of from 5° to 10° C. and second with 48-hour cultures of the same strain cultivated for the same number of generations at 30° C. The readings of the reactions were made after one and one-half hours and were all found to be negative, no traces of agglutination appearing in any of the tubes. After sixteen hours the tubes were all examined again. At this time in those containing the suspensions which were made with the bacteria cultivated at 5° to 10° C. there was almost complete precipitation of the organisms, the overlying liquid being clear in all, including the control tubes with normal serum and those containing suspensions of the bacteria in saline solution with no serum, as well as the tubes with the immune sera. In the tubes which contained the serum and suspensions of the organisms

[66] *Loc. cit.*

cultivated at 30° C. there was almost no precipitation of the bacteria, the fluid still being cloudy. My experience has led me to conclude that the time limit of the agglutinative reaction with the plague bacillus should be placed at certainly not over one or two hours at 37° C. at the most, since after this time the results may be very inaccurate and confusing owing to the tendency of the bacteria to become spontaneously precipitated.

The testing of serum supposed to contain relatively small amounts of plague agglutinins has been very unsatisfactory where it was necessary to employ concentrated mixtures of the serum, for example in dilutions of 0.5 or 0.25. As already mentioned, the density and viscosity of the serum in these dilutions evidently somewhat retards the agglutination of the pest organism, as was evident from the fact that the reaction occurred in some of the experiments in the higher but not in the lower dilutions. This phenomenon of retardation can not be explained on the assumption of the presence of agglutinoid, since it was noted in several instances with perfectly fresh sera. It may sometimes be observed in fresh, specific immune sera with other bacteria and must be ascribed to physical causes, the discussion of the nature of which will not be entered into here.

I have concluded from a large number of experiments that the agglutinins are formed slowly and only in very small amounts in animals which are being immunized against pest infection and that they only occur in demonstrable quantities in those which have been very highly immunized. At most, only very minute traces of these substances are encountered after single inoculations of either the killed or the living organisms, no matter how large the dose which is employed. I am convinced that in my earlier experiments with plague agglutination I sometimes mistook pseudo-agglutinations of the pest bacillus for true ones, and from a study of the literature it seems to me very likely that other observers have also erred in this respect. A study which I have made of the blood sera of guinea pigs which have been vaccinated against pest infection and which have later shown themselves to be immune to lethal and multiple lethal doses of the pest bacillus has demonstrated that practically no traces of agglutinins exist in such sera. The same may be said of the sera of other animals immunized in a similar manner. Monkeys which have first been vaccinated with attenuated pest cultures or inoculated with killed cultures, and which have afterwards been shown to be immune to pest infection by the injection in increasing doses of from ½ oese to nearly 1 entire agar slant culture of the living virulent strain, still have developed in their sera practically no traces of agglutinins. Indeed it is very difficult to immunize monkeys to such a high degree that the blood of the animals shows the presence of plague agglutinins. In a series comprising twelve monkeys (numbers 1232, 1233, 1239, 1240,

1247, 1286, 1287, 1300, 1301, 1302, 1357, 1358), in which an attempt was made gradually to immunize these animals with the strain "Pest Virulent" up to such a degree that these anti-bodies would be demonstrable in their blood, only two were able to survive the inoculations when the immunization had reached the point in which the agglutinins could be detected even in small amounts. (Numbers 1300 and 1357. See agglutination experiments, p. 246.) The remaining ten animals succumbed either to pest infection or intoxication as a result of the injections, before agglutinins could be shown to exist in their blood. Moreover, notwithstanding the fact that from $\frac{1}{4}$ to $\frac{1}{2}$ oese of this strain "Pest Virulent" represented a certain lethal dose for normal animals of this species and although these monkeys were immunized to such an extent that in a number of instances they were able to resist and survive the inoculations of such large amounts as 6 to 8 oesen of this organism (over twelve times the maximum fatal dose), agglutinins were still not present in sufficient quantities to be demonstrable in their blood.

Only small quantities of agglutinins could be detected in the examination of several pest immune sera which were prepared from horses and which were known to possess, in two instances at least, considerable protective (anti-infectious) power.

A horse which was being immunized against pest and which had acquired a sufficient immunity to withstand the injection of nearly 10 agar cultures of a virulent pest strain, gave a serum which at this time showed an agglutinative reaction in dilutions of 1 to 10, but none above this strength. However, at the same time 1 cubic centimeter of this serum protected from fatal pest infection about 60 per cent of the white rats inoculated. (See Series 45), p. 284. A pest immune serum obtained from Asia, which protected about 72 per cent of the inoculated rats against fatal pest infection in doses of from 1 to 2 cubic centimeters (see Series 8, 10, and 20, pp. 274, and 281), when carefully tested with the virulent pest strain showed no agglutinative reaction after three hours. However, it must be stated that at the time its anti-infectious and agglutinative reactions were tested this serum had been bottled for about a year. A second pest serum purchased from Asia which showed a somewhat lower protective power (see Series 27, p. 283) also revealed no agglutination when tested with the strain "Pest Avirulent." This serum had been bottled about nine months. Moreover, a laboratory immune serum which possessed a higher anti-infectious power and which protected about 90 per cent of the inoculated white rats against plague infection (see Series 53, p. 286) in doses of 1 cubic centimeter also showed almost no agglutinative reaction against the virulent strain giving only a weak reaction in a dilution of 1 to 10.

The following selected experiments justify the preceding remarks upon agglutination. Experiments performed with a number of animals less

highly immunized and in which the blood also showed no agglutination will not be given. The reactions were all performed with the virulent strain, excepting in a few instances when it is so stated, and by the macroscopic method. One oese of the bacteria was suspended in 1 cubic centimeter, 0.85 per cent saline solution and 1 cubic centimeter of the serum to be tested, diluted with saline solution, was added to the suspension. It is perhaps unnecessary to state that the microscopic method of observing agglutination with the plague bacillus is entirely untrustworthy.

DETAILS OF THE EXPERIMENTS.

Monkey number 1231.—Vaccinated October 10 with 1 culture "Pest Avirulent." Reinoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle which had been dipped in a suspension of "Pest Virulent." On October 28, 1 oese "Pest Virulent" was inoculated subcutaneously; on November 16 the animal was killed, and the agglutination of the serum tested in dilutions 1: 2 and 1: 4; two controls of the reactions (of the bacterial suspension without serum) were performed. All were negative after one and four hours.

Rabbit number 1960.—May 11: One 48-hour slant culture of "Pest Avirulent" in 1 cubic centimeter of bouillon was injected intravenously. The agglutination was tested twelve days later in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320, two controls of the reaction (of the bacterial suspension without serum) were prepared. All the reactions were negative after one and three-fourths hours. After sixteen hours, pseudo-reactions in dilutions of 1: 20 to 1: 60 were observed, the bacteria being precipitated and the suspensions appearing clear; the two control reactions were negative. The serum was again tested on the following day in dilutions of 1: 40, 1: 80, 1: 160, with two controls. The reactions were all negative after one hour. After sixteen hours, pseudo-reactions in the dilution of 1: 40 were observed. Three days later the serum was again tested in dilutions of 1: 5 and 1: 10 and two controls (without serum). The results were negative after one and one-half hours. After sixteen hours pseudo-reactions in dilutions of 1: 5 and 1: 10 were observed; both controls were negative.

Normal rabbit's serum was tested on this same date in dilutions of 1: 5 and 1: 10, with two controls of the bacterial suspension. All were negative after one and one-half hours. After sixteen hours pseudo-reactions in dilutions of 1: 5 and 1: 10 were observed; both controls were negative.

Rabbit number 1961.—May 12: One 48-hour culture of "Pest Avirulent," suspended in 1 cubic centimeter of bouillon, was injected intravenously. Twelve days later the animal was killed and the agglutination tested in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320. Three controls of the reactions without serum and 1 control with normal rabbit serum in a dilution of 1: 30 were performed. After one hour pseudo-agglutination was observed in the dilutions of 1: 20 and 1: 40. The remaining tubes were all negative; after sixteen hours pseudo-reactions took place in dilutions of from 1: 20 to 1: 80. The same serum tested the following day in dilutions of 1: 2 gave no agglutination after one and one-half hours.

Rabbit number 1963.—Inoculated intravenously with 5 cubic centimeters of Haffkine's prophylactic. Five days after the inoculation the animal was killed and the agglutination tested in dilutions of 1: 2 and 1: 5, with two controls. The results were negative after two and sixteen hours.

Rabbit number 1965.—Inoculated with 5 cubic centimeters of Haffkine's prophylactic intravenously. Ten days later the animal was killed and the agglutination

tested in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320; three controls of the bacterial suspension without serum and two controls with normal serum in dilutions of 1: 10 and 1: 40 were prepared. After one hour all reactions were negative; after sixteen hours precipitation of the bacteria occurred in dilutions of 1: 20 and 1: 40 and in the normal serum in dilutions of 1: 10. The same serum tested the following day; in dilutions of 1: 2, gave a reaction after one and one-half hours.

Rabbit number 1967.—Two cubic centimeters of the autolyzed product obtained from 20 milligrams of the strain "Pest Virulent" were injected intravenously; the animal was killed ten days later and the agglutination in dilution of 1: 2 tested. The reaction was negative after one and one-half hours.

Rabbit number 1968.—Inoculated in the same manner as number 1967 and with the same results.

Monkey number 1278.—Inoculated subcutaneously October 19 with 1 agar culture of "Pest Avirulent;" on October 30 inoculated subcutaneously with 2 oesen of "Pest Virulent." On November 17 the animal was killed and the agglutination tested in dilutions of 1: 2, 1: 4, with two controls without serum. The reactions were all negative after four hours.

Monkey number 1285.—Inoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle dipped in a suspension of the strain "Pest Virulent;" on October 25 the animal was reinoculated with 2 oesen of "Pest Virulent;" on November 17 it was killed and the agglutination tested in dilutions of 1: 2 and 1: 4, with two controls of the bacterial suspension without serum. All reactions were negative after two hours.

Monkey number 1286.—October 20: The skin over the abdomen was scarified and a suspension of "Pest Virulent" rubbed over this area. On October 25, 1 oese of "Pest Virulent" was injected subcutaneously; on December 2, $\frac{2}{3}$ of an agar slant of "Pest Virulent" was given subcutaneously; on December 14, $\frac{2}{3}$ of an agar slant of "Pest Virulent" was injected subcutaneously; on December 16 the animal died. The serum was tested on November 17 in dilutions of 1: 2 and 1: 4. Both were negative after two hours.

Monkey number 1287.—Inoculated exactly as monkey number 1286 up to December 14; the animal died on December 15, the day after the last inoculation. The serum was tested on the following day in dilutions of 1: 2 and 1: 4; the result was negative after two hours.

Monkey number 1300.—October 25, two agar slant cultures of "Pest Avirulent" injected subcutaneously; November 10, $\frac{1}{2}$ oese "Pest Virulent;" on December 2, 2 oesen of "Pest Virulent" were given; on December 14, 4 oesen "Pest Virulent;" on February 15, one 48-hour agar slant of "Pest Virulent" and on February 27, two 48-hour agar slants "Pest Virulent" were injected. March 10 the agglutination was tested in dilutions of 1: 2 and 1: 10. After two hours there was positive agglutination in dilutions of 1: 2, the fluid being almost entirely clear. A slight precipitation of the bacteria was observed in the dilution of 1: 10.

Monkey number 1357.—November 2, infected with a 10 cubic centimeter syringe needle dipped in a suspension of "Pest Virulent;" on December 2, $\frac{1}{3}$ of an agar slant of "Pest Virulent" was inoculated subcutaneously; on December 14, $\frac{2}{3}$ of an agar slant subcutaneously; on January 10, 1 agar slant of "Pest Virulent;" on February 1, $1\frac{1}{2}$ agar slants of "Pest Virulent;" on February 27, 2 agar slants of "Pest Virulent;" on March 15, 3 agar slants of "Pest Virulent." The animal died on March 17. The agglutination in the dilutions of 1: 5 was positive, the fluid being clear after one hour; in the dilution of 1: 10 there was a positive weak reaction after four hours.

Monkey number 2666.—Inoculated October 27. One 48-hour culture of *killed* "Pest Virulent" being given subcutaneously; on November 28, reinoculated with

⅔ oese "Pest Virulent;" on December 28 with 9 cultures of killed "Pest Virulent," subcutaneously. On January 10, the animal was killed. The agglutination tested in dilutions of 1: 5, 1: 10 and 1: 20 were all negative after two hours.

Monkey number 2720.—October 29, one agar culture "Pest Avirulent" injected subcutaneously; on November 28, ⅔ oese "Pest Virulent" given subcutaneously; on December 29, 1 oese of "Pest Virulent" given subcutaneously; on February 7 the animal was bled and the serum tested for agglutination. The serum of monkey number 2666 (see above) and of monkey number 3053 (normal serum) were also both tested on this date with 72-hour cultures of "Pest Virulent" grown in the ice box for six generations and with cultures grown for the same length of time and for the same number of generations at 30° C.

The results obtained are as follows:

TABLE No. I.—*Serum of immune monkey number 2666—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

[The term "control" throughout the experiments signifies a suspension of the bacteria in saline solution without the addition of serum.]

Dilutions.	After 1½ hours.	After 16 hours.
GROWN IN ICE BOX.		
1:10	Negative	Heavy precipitate. Fluid clear.
1:20	do	Do.
Control.	do	Do.
Control.	do	Do.
GROWN AT 30° C.		
1:10	Negative	Negative. Fluid not clear. Practically no precipitate.
1:20	do	Do.
Control.	do	Do.
Control.	do	Do.

TABLE No. II.—*Serum of immune monkey number 2720—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

Dilutions.	After 1½ hours.	After 16 hours.
GROWN IN ICE BOX.		
1:10	Negative	Heavy precipitate. Fluid almost clear.
1:20	do	Heavy precipitate. Fluid clear.
Control.	do	Do.
Control.	do	Do.
GROWN AT 30° C.		
1:10	Negative	Slight precipitate. Fluid not clear.
1:20	do	Negative.
Control.	do	Do.
Control.	do	Do.

TABLE No. III.—*Normal serum of monkey number 3053—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

Dilutions.	After 1½ hours.	After 16 hours.
GROWN IN ICE BOX.		
1 : 10	Negative	Heavy precipitate. Fluid almost clear.
1 : 20	do	Do.
Control.	do	Do.
Control.	do	Do.
GROWN AT 30° C.		
1 : 10	Negative	Slight precipitate. Fluid not clear.
1 : 20	do	Do.
Control.	do	Do.
Control.	do	Do.

Guinea pig number 3031.—Inoculated subcutaneously January 29 with one-half 24-hour culture of "Pest Virulent" partially attenuated. The animal died February 11, thirteen days after its inoculation, of advanced pest pneumonia and pest bubo. Agglutinative values of the serum of this animal and of the serum of a normal guinea pig (number 3053) were tested in dilutions of 1: 5 and 1: 10; the reactions were negative after two hours. After three hours, a moderate precipitation occurred in all of the tubes; quite as marked in those without serum as in the tubes with normal serum and in those of the pest guinea pig serum.

Guinea pig number 2636.—October 26, one culture of "Pest Avirulent" given subcutaneously.

Guinea pig number 2648.—October 26, one culture "Pest Maassen Alt" given subcutaneously. On November 26 both animals were reinoculated *cutaneously* with 5 oesen of a suspension of "Pest Virulent;" on January 17 they were reinoculated cutaneously with a portion of the spleen of a guinea pig (number 2993) which had just died of pest; the abdomen being deeply scarified. On February 7, both animals were killed and the agglutination of their sera, together with normal guinea pig serum (number 3053), tested in dilutions of 1: 2 and 1: 4. The reactions were all negative after one hour; after sixteen hours a precipitation of the bacteria in nearly all of the tubes occurred.

Pest immune horse serum Number I purchased from Asia. This serum in doses of 1 to 2 cubic centimeters protected against fatal pest infection about 72 per cent of the rats inoculated. (See Series 8, 10, and 20, pp. 274, 275 and 281.) The agglutination was tested with the strain "Pest Virulent" (second transplant) in dilutions of 1: 2, 1: 5, 1: 10, 1: 20; controls with normal horse serum in dilutions of 1: 2, 1: 5, 1: 10, and two controls with saline solution without serum were also prepared. After three hours all the reactions were negative; after sixteen hours sedimentation occurred in the majority of the tubes. In none of the tubes was the sediment more marked than it was in one of the control tubes of the bacteria without serum.

Pest immune serum Number II (obtained from horse), its anti-infectious power (see Series 27) being somewhat lower than that of Serum Number I. The agglutinative value was tested with three strains of pest bacilli of different virulence; at the same time a normal monkey serum was tested for control purposes. The results obtained are as follows:

TABLE NO. IV.—*Foreign Serum Number II and normal monkey serum—Agglutination tests January 17.*

FOREIGN SERUM NUMBER II.

Dilutions.		After 1 hour.	After 1½ hours.	After 2 hours.	After 21 hours.
Virulent strain.	1:2	Negative	Slight precipitate.	Distinct precipitate.	Marked precipitate. Fluid not entirely clear.
	1:10	do	do	do	Very heavy precipitate. Fluid cleared.
	Control.	do	Negative	Negative	Negative.
Avirulent strain.	1:2	do	Positive		Heavy precipitate. Fluid cleared.
	1:10	Positive	do		Do.
	Control.	Negative	do		Do.

NORMAL MONKEY SERUM.

Virulent strain.	1:2	Negative	Negative	Negative	Negative.
	1:10	do	do	Bacteria beginning to sediment.	Heavy precipitate. Fluid not entirely clear.
	Control.	do	do	Negative	Negative.
Avirulent strain.	1:2		Positive		Moderate precipitate. Fluid not clear.
	1:10		do		Heavy precipitate. Fluid entirely cleared.
	Control.		do		Do.

From the above experiments we can conclude only that the precipitate of the bacteria is coarser grained and that it occurs somewhat earlier in the immune serum than in the normal monkey serum.

TABLE NO. V.—*Foreign Serum Number II and immune monkey serum—Agglutination tests January 18.*

FOREIGN SERUM NUMBER II.

Dilutions.		After 1½ hours.	After 2 hours.	After 5 hours.
Maassen strain.	1:2	Negative	Negative	Negative.
	1:10	do	do	Do.
	1:20	do	Sediment beginning to form	Positive weak.
	Control.	do	Negative	Negative.
	Control.	do	do	Do.
Virulent strain.	1:10	do	do	Do.
	Control.	do	do	Do.

IMMUNE MONKEY SERUM.

Maassen strain.	1:2	Negative	Negative	Negative.
	1:10	do	Positive*	Positive.
	1:20	do	do.*	Do.
	Control.	do	Negative	Negative.
	Control.	do	do	Do.
Virulent strain.	1:10	do	do	After 5 hours.*
	Control.	do	do	Negative.

*A sedimentation is occurring and particles are collecting in the fluid, strongly simulating agglutination.

Pest immune serum Number III from a horse in the process of immunization by Dr. Ruediger. The last injection before bleeding was with nearly 10 agar cultures of "Pest Virulent" intravenously. The serum was drawn and tested for agglutination fifteen days after this injection. A horse's normal serum was also tested at the same time. The results are as follows:

TABLE No. VI.—*Immune serum Number III and normal serum (for control)—January 23, agglutination with strain "Pest Virulent" second transplant, 24-hour cultures.*

Dilution.	After 1 hour.	After 4 hours.	After 20 hours.
IMMUNE SERUM NUMBER III.			
1:2	Negative	Positive	Positive.
1:10	Positive	do	Do.
NORMAL SERUM (FOR CONTROL).			
1:2	Negative	Negative	Negative.
1:10	do	do	Very slight sediment.
Control.	do	do	Positive.
Control.	do	do	Negative.

TABLE No. VII.

January 25, the same sera as in Table No. VI again tested, with the following results:

Dilution.	After 1 hour.	After 2 hours.	After 16 hours.
IMMUNE SERUM NUMBER III.			
1:10	Positive	Positive. Marked precipitate.	Positive fluid cleared.
1:20	Negative	Negative	Considerable sediment. Fluid cleared.
NORMAL SERUM (FOR CONTROL).			
1:10	Negative	Negative	Considerable sediment. Fluid almost cleared.
1:20	do	do	Do.
Control.	do	do	Negative. Slight precipitate.
Control.	do	do	Do.

The same sera were tested with a 24-hour culture of the strain "Pest Avirulent" on this date. The results were as follows:

TABLE NUMBER VIII.

Dilution.		After 1 hour.	After 2 hours.	After 16 hours.
Immune Serum.	1:10	Positive	Positive	Positive.
	Control.	Negative		Marked precipitate.
Normal Serum.	1:10	do	Marked flocculent precipitate.	Do.
	Control.	Negative		Do.

Agglutination tests were made of the same horse's serum, the animal having received 10 agar cultures intravenously before this examination. The anti-infectious power of the serum at this time was such that 1 cubic centimeter protected 60 per cent of the inoculated white rats against fatal pest infection. The results are as follows:

TABLE NO. IX.—*Immune serum Number III with strains "Maassen Alt" and "Pest Avirulent."*

"MAASSEN ALT."

Dilution.	After 4 hours.	After 20 hours.
1:10	Positive	Positive. Fluid cleared.
1:20	Negative	Moderate sediment. Fluid not clear.
1:40	do	Do.
1:80	do	Do.
Control.	Very slight precipitate of bacteria	Do.
Control.	Negative	Do.

"PEST AVIRULENT."

Dilution.	After 2 hours.	After 4 hours.
1:10	Positive	Positive. Fluid cleared.
1:20	Negative	Do.
1:100	do	Negative, Slight sediment.
1:1,000	do	Do.
1:2,000	do	Do.
Control.	do	Sediment. Fluid not clear.
Control.	do	Do.

The agglutination of *Pest immune serum Number IV*, obtained from a horse, was tested five months after its preparation. Its anti-infectious value was such that 1 cubic centimeter protected 90 per cent of the inoculated white rats against pest. The agglutination tests were made with the strain "Pest Virulent" (second transplant) at this time in dilutions of 1:10, 1:20, 1:100, 1:500, 1:1,000, with two controls. All were negative after four hours except in the dilution of 1:10, where a weak positive reaction occurred.

THE AGGLUTINATING PROPERTIES OF THE BLOOD IN IMMUNIZED HUMAN BEINGS.

A study of the agglutinating properties in the blood of a number of persons who had been vaccinated against plague, as well as of several cases who had suffered with the disease, is summarized in the following table. The blood was collected and tested from the vaccinated cases, from one week to ten days after the inoculation. It was taken from those convalescent from plague ten days after the subsidence of the fever. In the two cases in which it was obtained at autopsy, the patients had lived ten and fourteen days, respectively, after the onset of the disease. In order that the tests might be carefully and repeatedly made, from 10 to 20 cubic centimeters of blood was collected in the living cases from a vein of the arm. In the majority of instances reactions were at the same time performed with fresh, normal human serum. The sera were all tested within a day or two after the blood was drawn. A second transplant 24-hour culture of the virulent strain was employed in all the tests.

TABLE No. X.—*Tests of the agglutinating value of human immune sera.*

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
I	$\frac{1}{10}$ oese "Pest Avirulent;" 1 week later $\frac{1}{2}$ oese "Pest Avirulent."	1:2; 1:5; 1:10; 1:20; 2 controls (no serum).	After 1 hour all negative. After 16 hours the precipitation of the bacteria is quite marked in all of the tubes but not so marked or of so dense a nature in the control tubes.
I	----do----	1:2; 1:5; 1:10; 1:20; 1:50; 1:100; 2 controls (no serum).	After 1 hour all negative. After 16 hours sediment in all the tubes but more marked in the tubes containing serum than in the control tubes. The same sera tested again the following day with the same results.
		1 control horse pest immune serum.	Positive after 1 hour.
II	$\frac{1}{2}$ oese "Pest Avirulent."	1:10; 1:20; 4 controls (no serum).	After 1 hour all negative. After 16 hours sediment in all the tubes; more marked in all tubes containing s rum than in control tubes. The same sera were tested on the following day with similar results.
		2 controls with normal human serum, dilutions 1:10; 1:20.	
III	2 oesen "Pest Avirulent."	1:10; 1:20; 1:40; 1:80; 1:160; 2 controls without serum.	After 2 hours distinct precipitation of bacteria in dilution of 1:10; after 2½ hours precipitation appears also in dilutions of 1:80 and 1:160. Remaining tubes show no precipitation.
		1 control normal human serum, 1:20.	

TABLE No. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
IV	2 oesen "Pest Avirulent."	1:10; 1:20; 1:40; 1:80; 1:160; 2 controls without serum.	After 2 hours faint precipitation of bacteria in dilution of 1:10. After 3 hours the same precipitation appears in the dilution of 1:20. The control tube with normal serum and the remaining tubes show no precipitation at this time.
		1 control normal human serum, 1:20.	
		1:2; 1:10; 2 controls (no serum).	Moderate precipitation of bacteria after 2 hours in dilutions of 1:2 and 1:10. No sediment in control tubes.
V	do	1 control normal human serum, 1:5.	Slight sediment of bacteria after 3 hours.
		1 control horse pest immune serum, 1:10.	Positive after 1 hour.
V	do	1:10; 1:20; 1:40; 1:80; 3 controls (no serum).	After 2 hours marked precipitate of bacteria in dilutions of 1:10 to 1:40. Controls negative.
		Control normal human serum 1:10.	Negative after 2 hours.
VI	do	1:2; 1:10; 1:20; 1:40; 1:80; 2 controls (no serum).	After 2 hours marked precipitate of bacteria in dilutions of 1:2 to 1:40.
		Control normal serum 1:10.	Negative after 2 hours.
VII	do	1:2; 1:10; 2 controls (no serum).	After 2½ hours marked precipitate of bacteria in dilution of 1:2 and 1:10. Controls negative.
		Control normal human serum 1:5.	After 3 hours moderate precipitate of bacteria.
		Control horse pest immune serum 1:5.	Positive after 2 hours.
VII	do	1:10; 1:20; 1:40; 1:80; 1:160; 3 controls (no serum).	After 1 hour marked precipitate of bacteria in dilution of 1:40. After 2 hours marked precipitate in dilutions of 1:10, 1:20 and 1:80. Controls negative.
		Control normal human serum 1:10.	Negative after 2 hours.
		Control horse pest immune serum, 1:5.	Moderate precipitate after 2 hours.

TABLE No. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
VIII	One agar slant "Pest Avirulent."	1:5; 1:10; 1:20; 1:40; 1:80; 1:160; 1:320; 3 controls without serum.	After 2 hours no precipitate of bacteria in any of tubes. After 16 hours marked precipitate in dilutions of 1:5, 1:20, and 1:40.
		Control normal human serum, 1:5; 1:10; 1:20; 3 controls without serum.	After 2 hours moderate precipitation of bacteria in dilution of 1:10; after 4 hours in dilutions of 1:5 and 1:20.
VIII	----do----	1:2; 1:5; 1:10; 1:20; 5 controls without serum.	After 2 hours all negative. After 16 hours marked precipitation with clearing of fluid in dilutions of 1:5, 1:10 and 1:20 Not in 1:2.
		Control with human serum, 1:5; 1:10; 1:20. 3 controls (no serum).	After 2 hours all negative. After 16 hours marked precipitation with clearing of fluid in dilutions of 1:5, 1:10, and 1:20. Controls negative.
IX	----do----	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. After 16 hours dilution 1:5 marked precipitation. Other tubes negative.
		Control normal human serum I, 1:2; 1:10.	Negative after 1 hour. After 1½ hours flocculent precipitate strongly resembling agglutination appears in the dilution of 1:10 of one normal serum and in the dilution of 1:5 in the other. Precipitate disappears on shaking. Dilutions of 1:2 with each serum negative. After 15 hours dilutions of 1:10 and 1:5, heavy precipitate. Dilutions of 1:2 still negative.
		Control normal human serum II, 1:2; 1:5.	
X	----do----	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. After 16 hours moderate precipitate of bacteria in dilution of 1:5.
		Control normal human serum, 1:1; 1:5.	Negative after 1 hour.
X	----do----	1:2; 1:5; 2 controls without serum.	Negative after 1 hour. Moderate precipitate of bacteria in dilution of 1:5 after 16 hours.
		Control normal human serum, 1:2; 1:5.	Negative after 1 hour. Moderate precipitation of bacteria in dilution of 1:5 after 16 hours.
XI	----do----	----do----	Exactly the same results obtained as in Case X.
XII	----do----	1:2; 2 controls without serum.	Negative after 1½ hours.
XIV	----do----	1:2; 1:5; 2 controls without serum.	Negative after 1 hour.

TABLE No. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
XV	One agar slant "Pest Avirulent."	1:2; 1:5; 2 controls (no serum).	After 1 hour and 10 minutes a flocculent precipitate began to appear in the dilution of 1:10. (Compare with Case IX reaction with normal serum.) Dilution 1:2 negative.
		----do----------	All negative after 1 hour. After 16 hours moderate sediment in dilution of 1:5.
XVII	----do----------	Control normal human serum, 1:2; 1:5.	Both negative after 1 and 16 hours.
XVIII	-----do----------	1:2; 1:5; 2 controls (no serum).	Exactly the same results as with Serum XVII.
XIX, XX, XXI, XXIII	----do----------	-----do----------	All negative after 1 hour. In Case XXI slight precipitate in dilution of 1:5 after 3 hours.
		----do----------	All negative after 1 hour.
XXIV	-----do----------	Control normal human serum, 1:2; 1:5.	Negative after 1 hour. Slight precipitate in dilution of 1:5 after 3 hours.
XXV	-----do----------	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. Sediment in dilution of 1:5 after 16 hours.
XXVI, XXIX, XXXI, XXXIII	----do----------	-----do----------	All negative after 1 hour.
Plague case	----------	-----do----------	Do.
Plague autopsies I and II.	----------	-----do----------	Do.

A careful study of the above table brings us to the conclusion that in the majority of the instances no traces of agglutinins could definitely be demonstrated. In a few cases they perhaps were present in very small amounts.

In concluding the remarks on agglutination it may again be emphasized that excessive precautions must be taken to distinguish between pseudo and true agglutination in pest, and that apparently only in the organism highly immunized against plague infection do the agglutinins become developed in sufficient quantity to be of any practical importance either in the diagnosis of the infection or in the demonstration of the presence of an immunity. It also would appear from these experiments that the development of the anti-infectious substances in a plague immune serum is quite independent of the development of the agglutinins.

IX. THE BACTERICIDAL ACTION OF PLAGUE SERUM.

The German Plague Commission concluded that in pest immune sera specific bactericidal anti-bodies were present, the action of which was fully analogous to that of the protective substances which had been demonstrated by R. Pfeiffer to exist in cholera and typhoid infection. However, they made no experiments which demonstrated that such sera possessed a bactericidal action against the pest bacillus, although some experiments were performed which demonstrated their preventive action against infection and their curative value. Nevertheless, the opinion that plague immune serum exerts a bactericidal action against this organism apparently became generally accepted, although but little experimental work was carried on upon the subject.

In 1902, Kolle and Martini [67] performed experiments with guinea pigs and rats in which the animals were inoculated with from 1 to 2 cubic centimeters of pest immune serum and after twenty-four hours were inoculated intraperitoneally with from 2 to 3 oesen of pest cultures of moderate virulence, suspended in saline solution. Three or four hours after the inoculation of the bacteria, upon microscopical examination according to the method of Pfeiffer, of drops of the exudate from the abdominal cavity, the majority of the bacilli were found to be swollen, degenerated, and broken up. This phenomenon was not noted in control animals treated with normal serum, but pest bacilli of normal appearance were observed, which increased in number from hour to hour up to the time of the death of the animal. The abdominal exudate of the animals injected with immune serum was sometimes apparently sterile after twenty-four hours, although in these cases the few remaining bacteria usually multiplied and caused the death of the animal at a later time. Animals, such as rats, which had been actively immunized against pest by repeated subcutaneous injections of living, attenuated cultures, when injected intraperitoneally with pest strains of moderate virulence also exhibited the same bactericidal action toward the bacteria, but no anti-toxic action could be observed.

Markl [68] as a result of his observations found that the process of destruction of pest bacilli varied according to the virulence of the organism. When a culture of very great virulence was inoculated into the abdominal cavity of a guinea pig which had been treated with an immune serum, after thirty minutes a very extensive leucocytosis occurred and the bacteria were taken up by the phagocytes. Those bacteria which remained free, became agglutinated and grouped about the leucocytes. One hour after the injection of the immune serum he could find no extracellular bacilli in the peritoneal exudate and cultures made from it either remained sterile or developed only a few colonies. A leucocytosis also occurred in control animals without immune serum, but the bacilli remained in this

[67] *Deutsche med. Wchnsch.* (1902), **28**, 45.

[68] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **42**, 244.

instance extracellular and cultures of the abdominal exudate on agar produced a rich growth of bacteria. In further experiments on rats, where strains of different pathogenetic power were employed, the very virulent strains were taken up by the phagocytes through the action of the immune serum, while avirulent strains (those non-lethal in doses of 2 oesen) became dissolved in the abdominal cavity of the animal without the aid of the phagocytes. Strains of moderate virulence became partially destroyed by both of these means. The same mechanism was observed in passive immunization with serum, as was seen in animals actively immunized, either with killed or with living attenuated cultures.

Kolle[69] in December, 1904, on pursuing further experiments of this nature, confirmed Markl's results. He also compared the method of action of plague, cholera and typhoid immune sera, testing the bactericidal action of plague serum *in vitro* after the method of Neisser and Wechsberg. He was unable to demonstrate any bactericidal reaction, in spite of the many variations in the experiments and the use of many different sera to supply the complement for the completion of the action of the amboceptors. Plague bacilli after treatment with the immune serum developed as plentifully in the culture media as they did in those instances in which they were treated with normal serum. Kolle and Hetsch employed fresh, normal serum from the pigeon, cow, horse, chicken, rabbit, donkey, and rat for supplying the complement. Upon investigating the question of whether the bacterial receptors of the plague bacillus were able to bind amboceptors in an immune serum, it was shown that such a binding did actually take place, and that the serum after being first treated with living plague bacilli lost in anti-infectious power, as was shown by experiments performed on rats. However, at the temperature of the ice box or when killed pest bacilli were substituted for living ones, binding of the amboceptors could not be demonstrated. Moreover, the union did not occur under the same quantitative relations as it does with typhoid and cholera immune serum. Kolle therefore concluded that the pest serum acts neither as a pure antitoxic serum, such as we see in diphtheria and tetanus, nor as a pure bactericidal one, such as we see in cholera and typhoid.

Skschivan[70] also obtained Pfeiffer's phenomenon in guinea pigs which were inoculated with 4 cubic centimeters of the Paris pest serum and sixteen to twenty hours later reinoculated with from 2 oesen to 1 agar culture intraperitoneally. When the serum was inoculated into the peritoneum, the bacteria became broken up in one-half hour. The control animals without serum died after one to two days while those inoculated with immune serum lived for from five to seven days. In the latter instance, in three cases, a periorchitis existed and the omentum was shrunken and hard.

In 1902, Wright and Windsor[71] showed that normal human serum was entirely without bactericidal action upon the pest bacillus and that sterilized cultures of this organism were not capable of abstracting a bactericidal element from such blood. In the following year Wright and Douglas[72] further showed that, while no bactericidal reaction was exerted against the plague bacillus by normal human serum, such serum evidenced a distinct opsonic action against this organism. On the other hand Row[73] maintained that the serum of plague convalescents possessed remarkable bactericidal properties. He found that while in hanging.

[69] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 371.
[70] *Centrbl. f. Bakteriol. Orig.* (1903), **33**, 271.
[71] *J. of Hyg.* (1902), **2**, 385.
[72] *Proc. Roy. Soc. Lond.* (1904), **73**, 130.
[73] *Brit. Med. Journ.* (1902), 1895.

drop preparations, the *Bacillus pestis* flourished in the serum of normal individuals, when a quantity of serum from a plague convalescent was infected with this organism the latter was destroyed after twenty-four hours. The growth of the plague bacillus was said to be inhibited, in the case of blood taken from patients in the early stage of the disease or from those tending to recover. In a later paper [74] he pointed out that this bactericidal reaction of the pest bacillus resulted from the action of immune bodies plus complement, and that the action of plague serum could be suspended by the usual method of inactivation and it could again be reactivated by the addition of fresh complement. He claimed that Roux's serum which had been inactivated, could be reactivated by fresh complement obtained from either man, the dog, rat, or monkey. However, Row's experiments seem inconclusive and unconvincing owing to the technique employed.

In 1906, Lamb and Foster [75] again emphasized the fact that not only normal human serum, but also that of other mammals, was devoid of any bactericidal action on *Bacillus pestis*.

Schouroupoff,[76] who demonstrated that the pest bacilli which were injected into animals immunized against pest, disappeared after a very short time, concluded that the antipest serum is also partly bactericidal in its action.

Löhlein [77] very recently communicated the results of his studies upon phagocytosis in pest and anthrax to the Association of Microbiology at Berlin. He found that while in the animal body almost no phagocytosis of virulent pest strains occurred, *in vitro* the organisms were taken up by the carefully washed white blood corpuscles of the most susceptible experimental animals and he further demonstrated that while phagocytosis of the virulent pest organism was not dependent upon the action of dissolved opsonic substances, such action was promoted by normal guinea pig serum. The same phenomena were observed with dilutions of specific plague serum. In the case of undiluted serum, or in its lower dilutions, the serum was strongly agglutinative, so that the result was obscured. Lohlein, in accord with the experiments of Markl, found that *in vitro* the most marked prevention to the growth of virulent pest bacilli occurred when immune serum and leucocytes were placed in contact with the pest bacilli. Upon injecting virulent pest germs into the "prepared" abdominal cavity of guinea pigs, a marked phagocytosis of the bacteria also occurred. A rapid diminution of the bacteria at first, as well as of the animal cells in the abdominal cavity, also occurred upon injecting these organisms into the abdominal cavity of a *normal* guinea pig or rat. A dissolution of the bacteria was not observed, but the organisms were taken up by phagocytic cells in different portions of the abdomen and in the omentum. Later, a marked increase in the number of leucocytes in the abdominal cavity took place and especially, the bacteria became increased in number. These organisms, which were termed "bacteria of the second generation," increased up to the time of the death of the animal. The bacteria which appeared after the "negative" period were not usually taken up by phagocytes. This failure of the leucocytes to ingest them was evidently not due to an injury to the phagocytic properties of these cells, as was demonstrated by the further injection of staphylococci into the abdominal cavity, the latter organisms undergoing active phagocytosis. Lohlein thought this phenomenon might be explained either upon the assumption of the presence of a soluble

[74] *Brit. Med. Journ.* (1903), 1078.
[75] *Lancet* (1906), **171**, 9.
[76] *Arch. des Sci. Biologiques* (1905), **11**, 196.
[77] *Centrbl. f. Bakteriol. Abt. I* (1906), **38**, 32.

substances such as an aggressin, in the sense in which Bail uses the term, or by assuming that the bacteria which appeared later in the abdominal cavity, surrounded by capsules, possessed a negative chemotactic action. Possibly both of these factors played a rôle, although he was unable to determine the question definitely. He also found, as Denys and Tartakowsky had done, that where immune serum was injected into the abdominal cavity of a guinea pig, shortly after the introduction of the organisms, phagocytosis in addition to agglutination of these bacteria of the second generation occurred.

After a study of the experiments which had been performed in relation to the bactericidal action of the pest immune serum *in vitro,* it appeared to me, to judge from the careful experiments of Kolle and Hetsch on pest immune serum, that this question was definitely settled as far as the methods employed were concerned. The experiments of Wright and Lamb and my own already referred to, relating to the bactericidal reaction *in vitro* of normal human and other mammal's blood, also appeared conclusive. However, in order further to complete the evidence, the following additional experiments, consisting of the examination of the bactericidal power of horse's perfectly fresh pest immune serum, as compared with horse's fresh normal serum, were undertaken. I also studied the bactericidal power of horse's inactivated pest immune serum, after the addition of perfectly fresh monkey serum to supply the complement. The horse's immune serum in the latter instance was inactivated by heating for one hour at 56° C. The experiments were performed in the following way:

In test tube I were placed 1 cubic centimeter of perfectly fresh, horse's immune serum (without the addition of carbolic acid) + 1 cubic centimeter of a suspension of an agar culture of the virulent pest bacillus in bouillon, in a dilution of 1 to 50,000.

In test tube II were placed 1 cubic centimeter of perfectly fresh, normal horse serum + 1 cubic centimeter of a suspension of an agar culture of the virulent pest bacillus in bouillon in a dilution of 1 to 50,000.

After thorough mixing of the contents of each tube they were both placed for three hours in the incubator at 37° C., when sets of agar plates were prepared. No difference in the bactericidal action of the two sera could be distinguished.

In the second series of experiments, in test tube I were placed 1 cubic centimeter of the inactivated, horse's immune serum + 0.5 cubic centimeter of the suspension of the bacilli + 0.5 cubic centimeter of monkey's fresh normal serum in a dilution of 1: 10.

In test tube II were placed 1 cubic centimeter of horse's inactivated, normal serum + 0.5 cubic centimeter of a suspension of the bacilli + 0.5 cubic centimeter of monkey's fresh, normal, serum in a dilution of 1: 10.

In test tube III were placed 0.5 cubic centimeter of the supension of the bacilli + 1.5 cubic centimeters of saline solution.

In test tube IV, 0.5 cubic centimeter of a suspension of the bacilli + 0.5 cubic centimeter monkey's normal serum + 0.5 cubic centimeter saline solution.

These experiments were performed in dilutions of the serum of 1: 2, 1: 10, 1: 100, and 1: 500. No marked difference could be discovered in the number of colonies in the plates prepared from the suspensions of immune serum and from that of the normal serum.

The success of the reaction in experiments of this nature, providing of course that the serum to be tested possesses a bactericidal power, obviously depends upon the selection of an animal serum containing complement with receptors suitable to unite with the amboceptors in the immune serum. As has been mentioned, Kolle and Hetsch in their experiments employed the sera of the pigeon, cow, horse, chicken, rabbit, donkey, and rat to furnish the complement.

However, it was demonstrated by the following additional experiments that my failure, in the experiments which I have just described, to obtain a bactericidal reaction or to demonstrate any other injurious effect on the plague bacillus resulting from the action of the pest immune serum on this organism was not due to the fact that I had not found a suitable complement for the amboceptors.

A horse's pest immune serum (not inactivated), *which at the time in doses of 1 cubic centimeter was able to protect against fatal pest infection about 90 per cent of the rats inoculated with it,* was mixed with perfectly fresh rat serum and its bactericidal value tested according to the method, *in vitro,* which has already been described. In order that the phenomenon of the deflection of the complement by amboceptors, as described by Neisser and Wechsberg, might not interfere with the reaction, the experiments were also performed with varying amounts of the horse's immune serum and fresh rat serum. However, no difference could be detected between the results obtained with these experiments and with those performed in the same manner with normal horse serum plus fresh rat serum.

These experiments and those of Kolle and Hetsch already outlined appear to demonstrate that the pest immune serum which is known to possess anti-infectious power in the animal, possesses *in vitro* no bactericidal action whatever, that is similar to that possessed, for example, by typhoid immune serum. It is also clear that the pest bacilli are not only not killed by the immune serum, but that they remain alive and are capable of subsequent development. Therefore, it is evident that some other factor must play an important rôle in the ultimate destruction of the inoculated bacilli in the body of an animal either passively immunized by the injection of such a serum or actively immunized by vaccination, and according to the conceptions of Metchnikoff it might be argued that since the serum in the test tube is apparently without injurious action upon the pest bacilli, the phagocyte must be the additional factor which is necessary to render harmless and to destroy the organism in question.

However, before accepting this hypothesis, it seemed advisable further to investigate not only what action the serum has upon the life of the plague organism, but also what action the organism has upon the

immune serum. From the extensive work performed by Ehrlich, Morgenroth, Pfeiffer, Wassermann, Kolle, and their co-workers, we know that a union occurs *in vitro* between the specific substances of a serum, such as antitoxine or bacteriolysine, and the homologous bacterial antigen. Although the union of these two substances follows a different law, it is possible to show that such a binding actually does take place and that the antitoxic serum loses in value after combination with toxine and the bactericidal one diminishes in its specific effect after treatment with its corresponding bacterium.

With a view of further elucidating the subject, this problem was undertaken with the plague bacillus and pest immune serum; the experiments being performed in a somewhat similar manner to those previously carried on in this laboratory in 1904 with the cholera organism. The pest immune serum was first carefuly tested for its anti-infectious power on rats and the amount determined which would protect about 90 per cent of the animals inoculated with it, against the subsequent injection of a lethal dose of plague bacilli. (See Series 53, p. 286.) Fifteen cubic centimeters of this pest serum were then mixed with the living bacteria obtained from fifteen 48-hour agar slant cultures of the virulent pest strain. The mixture was placed in the incubator for two hours at 37° C.; carbolic acid to 0.5 per cent was then added and the mixture heated for two hours at 46° C. and then thoroughly centrifuged. The clear fluid above was then drawn off from the sediment of bacteria. After the sterility of the serum had been demonstrated, its anti-infectious value was now for a second time tested on rats and it then was found that the serum no longer protected these animals in the same amounts as it did previous to its treatment with the baceria, 70 per cent of the rats inoculated with the same dose succumbing when subsequently infected with pest.[78]

It follows from this that a binding of at least a portion of the amboceptors of the pest immune serum to the receptors of the plague bacillus had occurred, and although the bacteria in question were not killed by the serum, nevertheless, a reaction *in vitro* between the serum and the organism had occurred. (For the details of these experiments see Series 53, p. 286.) These results are confirmatory of those already referred to and obtained by Kolle and Hetsch.

For the further study of the action of plague immune serum I performed other experiments in the abdominal cavities of guinea pigs, the details of which may be seen in Series 63, 64, and 65 (pp. 268, 270, and 271). Upon injecting the strain "Pest Virulent" into the peritoneal

[78] In performing experiments of this nature precautions must be taken to ascertain if sufficient amounts of dissolved receptors of the bacilli (artificial aggressins) remain in the serum to influence the course of the infection in the animal.

cavity of a guinea pig immunized against plague either by previous vaccination or by the injection of a dose of pest immune serum, it was found that Pfeiffer's phenomenon as observed in the case of the cholera organism in the cholera immune animal did not occur. The virulent organism in question did not undergo dissolution and only with the very avirulent strains ("Maassen Alt" and "Pest Avirulent") did the organisms finally become swollen or disintegrated. It is true that shortly after the inoculation of the virulent strain in the immunized animal, a disappearance of the bacteria from the abdominal cavity usually occurred, and that also at first but few animal cells were encountered in the abdominal exudate. Upon investigating the fate of the bacteria by killing the animals at different periods of time after the inoculation, it was found that shortly after the injection, both in the case of animals immunized against pest and in that of normal animals, the majority of the bacteria had been carried to or made their way to the walls of the cavity and particularly to the omentum, to the surface of which they had become adherent. Here many of them were taken up by the phagocytic cells. After a short period, the leucocytes became more abundant in the abdominal exudate and many of them were seen to contain bacteria. In many cases, in the immunized animal, the leucocytes seem to possess positive chemotaxis for the bacteria, judging from the manner in which the latter are grouped about them.

In the case of nonimmune animals the pest bacilli outside of the cells increase in number up to the time of the death of the animal. The majority of the bacteria that are found to exist free in the cavity after the negative phase, are short, bipolar staining bacilli which seem to possess capsules; a smaller number of large bacilli, frequently showing involution forms, are also encountered. After the negative phase in the case of the immunized animal, the leucocytes usually become much more numerous in the abdominal cavity. The phagocytosis of the bacteria continues, both by the cells in the omentum, and by those free in the abdominal cavity, until very few free bacilli remain. However, as has been mentioned, in the nonimmune animals the bipolar staining organisms, which do not appear to be taken up by the leucocytes, increase up to the time of the death of the guinea pig. (See Series 63, 64, and 65 (pp. 268, 270, and 271.)

Löhlein,[79] whose article was published while my experiments were in progress, also encountered these bipolar staining organisms. These he very appropriately terms "bacteria of the second generation," and he calls attention to the fact that they are seldom seen to undergo phagocytosis. This phenomenon as mentioned he explains either upon the assumption that some such substance as the aggressin of Bail may be present, or that

[79] *Loc. cit.*

perhaps the capsules of the bacteria act in a negatively chemotactic manner. My own idea is that these organisms are negatively chemotactic for the leucocytes, but not because they possess aggressins in the sense which Bail uses the term, but because they have not been changed chemically by the action of the immune serum—that is, these bacteria have either entirely escaped the action of the plague amboceptors, or a sufficient portion of their receptors have not been bound to enable the leucocytes to act positively chemotactic toward them and to engulf them; the phagocyte usually ingesting only the organism which has previously been affected by the immune serum; it is also possible that the encapsulated organisms are not readily hydrolyzed.

Therefore, it is obvious that *in vitro* the amboceptors of plague immune serum unite with the receptors of the organism and that in the body of the animal the process of destruction is carried on further by the phagocytes, which engulf the bacteria which have been so acted upon. It is also evident that the bacteria are not killed in the test tube by the immune serum alone. Does the leucocyte accomplish this action, or is the organism killed in the abdominal cavity by other influences before undergoing phagocytosis? That the latter hypothesis is not always true I have been able to demonstrate by transplanting to the surface of agar media loops of the abdominal exudate which contain phagocytes inclosing bacteria. The organisms under these circumstances have sometimes been observed to have increased within the cells and in some instances to have burst the leucocyte and partially to have escaped from it.

It therefore seems that after the bacillus has been prepared for the action of the leucocyte by the immune serum, the leucocyte does play a part in the digestion and ultimate destruction of the organism. On the other hand, that the phagocytes in nonimmunized animals do not to any extent take up plague bacilli seems equally clear and this opinion may be confirmed by the study in plague infections of smears or sections from plague buboes or abscesses in other portions of the body. Here, while many bacteria are encountered in the endothelial cells, but very limited or no evidence of phagocytosis by polymorphonuclear leucocytes is encountered.

The destruction of the plague bacillus is then effected by the immune animal in a manner partly in accord with the humoral theory of Buchner and partly in accord with the phagocytic one of Metchnikoff. The action of the serum in its protective effect upon the animal is neither antitoxic nor bactericidal, but has been termed anti-infectious; that is, it is a serum possessed with the power of preventing infection.

X. OPSONIC ACTION OF PLAGUE SERUM.

As is now generally known, Wright and Douglas[80] in 1903 proposed the term "opsonins" to designate the elements in the blood which modified the bacteria in a manner which rendered them a ready prey to the phagocytes. Their important article, at the time of its appearance, did not excite the attention which it merited, but in 1904 and 1905 they, together with Bullock,[81] undertook further studies relating to the variations of the opsonic content of the blood in certain infections and explained the practical results to be obtained from the observation of the opsonic index. These publications attracted wide attention. During the past year many articles have appeared relating to the presence of opsonins in different infections and of the practical results to be obtained by increasing the opsonic index through the injection of bacterial vaccines. While some of this work has been done by trained investigators, much of it has been performed by men who may be considered new to the field and it is questionable whether all of the claims of the *practical* results obtained with the various infections will be justified by more careful work in the future. There are many sources of error in determining the opsonic index, and the personal equation is of considerable importance when the method described by Wright is employed.

Among the more important articles on this subject which have appeared during the past year, besides those of Wright, may be particularly mentioned those of Hektoen[82] and his pupils and of Simon, Rosenow and Porter[83] in the United States, and of Muir and Martin in Great Britain.

A discussion of the entire subject can not here be entered into. Hektoen and Bullock believed that the opsonins might be regarded as distinct from the other anti-bodies. However, it does not seem to me that this has been at all *conclusively* established, and in my experimental work on the subject I have not been able to convince myself that in the "opsonic" action in plague we have to do with any other factors than the binding of the receptors of the bacteria by amboceptors of the serum and of the union of the complement in the usual way, as in bacteriolysis, and believe that it is in this manner that the bacteria are prepared for phagocytosis. However, obviously the complement does not always necessarily cause lysis, although it always enters into the reaction.

[80] *Proc. Roy. Soc. Lond.* (1903), **72**, 357.

[81] *Ibid.* (1904), **73**, 128, and *Lancet* (1905), **2**, 1598 and 1603. These articles contain the other important references to Wright's work.

[82] *J. Am. Med. Ass.* (1906), **46**, 1407, and *J. Infect. Dis.* (1906), **3**, 434.

[83] *J. Am. Med. Ass.* (1906), **47**, 1722.

It seems not unlikely that in some of the experiments performed in the study of the opsonins it is sometimes the action of the complement alone and sometimes, though more rarely, that of the immune body which has been considered to represent the opsonic action. The recent work of Simon [84] and his associates which argues against the specific character of opsonins, supports these views, as does also the work of Muir and Martin.[85] These latter authors have tested the three chief varieties of immune bodies (amboceptors), namely, (1) those obtained by the injection of red blood corpuscles, (2) serum, and (3) bacteria, and have found that in each case the combination of receptors plus immune bodies removes the opsonins of normal serum. Moreover, it seems to me that another very important factor in favor of the hypothesis that opsonic action consists of the binding in combination of amboceptors and complement is furnished by a study of the action of the plague bacillus and plague serum in relation to the so-called phenomenon of Bordet and Gengou [86] of the fixation of the hæmolytic complement.

I also have been able to show that plague immune serum constantly produces the phenomenon and we can therefore reason from this fact and from the work of Muir and Martin that some complement as well as amboceptor enters into the opsonic reaction in plague, since we know that the complement is only bound according to the phenomenon of fixation when a union of specific amboceptor and receptor has occurred and that, conversely, the phenomenon only takes place when the complement is actually bound. (See pp. 327 and 328 for the details of these experiments.)

However, leaving aside the discussion of whether the opsonins really represent new anti-bodies of a different character from those we are familiar with, we may certainly say that plague immune serum possesses opsonic power since it prepares the organisms for phagocytosis, and we may add that with the plague bacillus the result of the chemical reaction is somewhat different from that which is observed in true bacteriolysis of certain other microörganisms.

I have also investigated the question of whether the sera of human beings and animals vaccinated against plague develop a higher opsonic index against the plague organism than is possessed by normal human and animal sera. Some of the guinea pigs and monkeys in which the reaction was tested had been shown to resist plague infection after the vaccination. The blood was usually tested ten days after the vaccination or test of immunization. The method of experimentation has been as follows:

A non-immunized guinea pig is inoculated intraperitoneally with 10 cubic centimeters of a sterile suspension of aleuronat. After from twelve to sixteen

[84] *J. Exp. Med.* (1906), **8**, 651.
[85] *Brit. Med. Journ.* (1906), 1783.
[86] *Ann. d. l'inst. Pasteur* (1901), **15**, 289.

hours the animal is killed with chloroform. The abdominal exudate, if sterile, is collected and mixed with an excess of 0.1 per cent of ammonium oxalate solution in 0.85 per cent sodium chloride solution (as proposed by Simon,)[87] and thoroughly centrifuged. The packed corpuscles are then washed several times in excess of saline solution and each time recentrifuged. In performing the tests one volume of leucocytes plus one volume of a saline suspension of the pest bacteria plus one volume of the serum to be tested are thoroughly mixed and incubated for thirty minutes at 37° C. After this time smears are prepared and stained with methylene blue. Two hundred leucocytes were counted and the average number of bacteria ingested determined. Control experiments with normal sera of the same species of animal were prepared as well as a control showing the nonphagocytic power of the washed leucocytes. The method of counting the phagocytic cells as suggested by Simon rather than the number of bacteria in each phagocyte as recommended by Wright, appears somewhat simpler and the error of variation between repeated counts with it was in some instances not so great. However, which of these methods will eventually prove to be the more reliable in determining the actual opsonic index I am not prepared to state.

A few experiments were also performed in determining the opsonic action of vaccinated animals by another method which, however, was not found to be as satisfactory as the one already described.

In these experiments the animals to be tested were first inoculated intraperitoneally with a suspension of the strain "Pest Virulent" and after thirty-five minutes to one and one-half hours, drops of the abdominal fluid were drawn off and smears prepared and stained. The average number of bacteria contained in 50 leucocytes was then counted.

While the animals which had been vaccinated by this method usually showed an increased opsonic index over that of normal animals, frequently the number of leucocytes in the abdominal cavity as well as the number of bacteria was very small. The explanation of this phenomenon has already been discussed above. The difficulties in the technique were therefore increased and further experiments were not performed with this method because of the superiority of the first one described. In the experiments in Series 61 (p. 267) the opsonic index of the animals before vaccination was unfortunately not determined and hence the results are not as valuable for comparison.

Carrying out experiments in this manner, the details of which are described in Series 61 and 62 (pp. 267 and 268), it has been possible to demonstrate that the blood of an individual or animal vaccinated and immunized against pest usually shows an increased opsonic index. However, that this was not invariably the case may be seen from the tables given in Series 61 and 62. The opsonic power apparently varied somewhat according to the virulence of the strain employed, the index usually being higher with the avirulent strain. No attempt was made to demon-

[87] *Johns Hopkins Hosp. Bull.* (1906), 17, 27.

strate the negative phase of the reaction or to plot their curves, and as the normal opsonic action is known to vary considerably from time to time, this may account for the apparent absence of the increase of the index in some of the cases at the time of the examination of the serum. Moreover, the reaction may not in some instances have reached its maximum or it may in others have begun to decline. However, the general law may be determined from the study of the entire series of experiments.

In conclusion it may then be stated that plague immune serum acts neither as an antitoxic serum nor as do other bactericidal sera, but its action may be said to be anti-infectious and opsonic in nature.

EXPERIMENTS.

SERIES 61.—*Determination of the opsonic index in guinea pigs.*

The animals were inoculated with 1 cubic centimeter of a suspension of the strain "Pest Virulent" in saline solution. Thirty-five minutes to one and one-half hours after the injection, drops of the abdominal fluid were drawn off and smears prepared and stained. The average number of bacteria contained in fifty leucocytes was then counted. Unfortunately, the opsonic index was not determined before the animals were vaccinated and therefore the experiments were performed in normal as well as in vaccinated animals.

Animal No.	How immunized.	Opsonic index of strain "Pest Virulent" after 1½ hours.
1977 (vaccinated)	Vaccinated with 2 cultures "Pest Avirulent." Immunity tested later.	11.3
2273 (normal)		9
1980 (vaccinated)	Vaccinated with 2 cultures "Pest Avirulent." Immunity tested later.	8
2282 (normal)		4.6
2154 (vaccinated)	1 agar slant "Pest Avirulent." Immunity tested later	8
2162 (vaccinated)	do	5
2861 (normal)		8

SERIES 62.—*Determination of the opsonic index in human beings, guinea pigs and monkeys.*

This series comprised the examination of the opsonic index in both human beings and animals vaccinated against pest. The opsonic index was tested against the strain "Pest Virulent" both before and after the immunization. In a few instances, after immunization, the opsonic index was also tested with two other strains of the pest bacillus, as may be seen from the table. One-tenth cubic centimeter of the serum, 0.1 cubic centimeter of a thoroughly washed suspension of guinea pig's corpuscles, and 0.1 cubic centimeter of a suspension of the organism "Pest Virulent" were thoroughly mixed and the resulting suspension placed at 37° C. for thirty minutes. Smears were then prepared and stained, two hundred leucocytes being counted.

SERIES 62—Continued.

Number.	Opsonic index for "Pest Virulent" before inoculation.	Vaccinated with—	Opsonic index for strain "Pest Virulent" tested 10 days after inoculation.	Opsonic index for strain "Avirulent Manila" tested 10 days after inoculation.	Opsonic index for strain "Pest Avirulent."
Human case 55	0.4	One 24-hour agar culture "Pest Avirulent"	2		
Human case 56	.48	do	.7		
Human case 58	.89	do	.8		
Human case 59	.64	do	1.7		
Human case 60	.4	do	.7		
Human case 61	.52	do	1.4		
Human case 62	1.2	do	.9		
Human case 64	4.6	do	5.8		
Monkey 2666 (vaccinated).	.34	1 culture killed "Pest Virulent." Immunity tested later.	.7		
Monkey 2717 (vaccinated).	.64	One 24-hour culture "Pest Avirulent." Immunity later tested.	2		
Monkey 3061 (normal).	1.		.8		
Guinea pig 2634 (vaccinated).	.42	One 24-hour culture "Pest Avirulent." Immunity tested later.	1.8		
Guinea pig 2680 (vaccinated).	.6	1 culture of killed "Pest Virulent." Immunity tested later.	1.7		
Guinea pig 3062 (normal).	.5		.7		
Guinea pig 2636 (vaccinated).	.48	One 24-hour culture "Pest Avirulent." Immunity tested later.	.4	1	5.4
Guinea pig 2648 (vaccinated).	.6	One 24-hour culture of "Maassen Alt." Immunity tested later.	2.4	3.2	1.8
Guinea pig 2639 (vaccinated).	.43	One 24-hour culture "Pest Avirulent." Immunity not tested.	.9	1.2	1
Guinea pig 3053 (normal).	.51		.4	.46	.62
Guinea pig 2231 (normal).	1.8		2		
Guinea pig 1975 (vaccinated).	1.4	Two 24-hour cultures "Pest Avirulent." Immunity tested later.	4.3		
Guinea pig 1976 (vaccinated).	2	do	3.1		

SERIES 63.—*Experiments relating to the mechanism of the action of plague immune serum.*

Guinea pigs numbered 2152 and 2153 which had been immunized by plague vaccination and numbers 2757 and 2758, which were not immunized, all received intraperitoneally 1 full oese of the strain "Pest Virulent" suspended in 1 cubic centimeter of saline solution. Guinea pigs numbered 2152, 2153, and 2757 each also received intraperitoneally, at the same time, 1 cubic centimeter of pest immune serum, and guinea pig number 2758, 1 cubic centimeter of saline solution. Drops of exudate were taken from the abdominal cavity at intervals of from twenty minutes to one and one-half hours after inoculation. Some of these

SERIES 63—Continued.

were dried and stained and others were examined in a fresh condition. On microscopic examination there was no evidence whatever of bacteriolysis either in the fluid from the animals immunized and inoculated with immune serum or in that from the control animal (number 2758) although there was a diminution in the number of the bacteria which had been inoculated in the exudates from all. Those organisms which were present stained naturally and presented no evidence of degeneration.

The leucocytes were much more numerous in the preparations from animals numbered 2152 and 2153, fewer in number in those from animal number 2757 and still more scanty in those from animal number 2758. At a later period a still more marked reduction of the number of the bacteria in the abdominal exudate from all the animals occurred. This reduction, however, was more marked in the animals which had received the serum. On the following morning the guinea pigs were all killed with chloroform and the abdominal cavity in each instance immediately opened and its contents examined. In guinea pig number 2152 there was almost no local reaction apparent about the point of inoculation. The abdominal cavity contained a small amount of a tenacious fluid. Cover slips from this exudate stained with methylene blue showed a considerable number of polymorphonuclear and large mononuclear leucocytes, the latter variety predominating. None of the leucocytes contained any bacteria and no bacteria were found free in the exudate. A small portion from the edge of the omentum was cut off and rubbed over a glass slide and the preparation stained with methylene blue. This specimen showed innumerable polymorphonuclear leucocytes and large, mononuclear, endothelial phagocytes, the majority crowded with pest bacilli. There were a number of free organisms, but these were swollen and frequently stained irregularly and usually faintly so in color. They showed no capsules and no bipolar staining. Many appeared as involution forms. It seems likely that a number of these organisms have been extruded from cells or that the cells have burst and become destroyed and the bacteria have escaped. This opinion is supported by the fact that many bacilli with a similar appearance were seen within degenerating cells or lying about them.

It appears evident that the bacteria have become acted upon in some manner by the immune serum so that the cells of the omentum to which the bacteria have been attracted are enabled to pick them up in large numbers. It would seem that the great reduction in the number of bacteria in the abdominal cavity during the first hour after inoculation is not due to the fact that the bacteria have been destroyed but because they have been attracted to the omentum as to a sponge. That the bacteria inside the leucocytes were not all dead was demonstrated by placing some of the leucocytes containing ingested bacilli upon agar cultures. It was then found that when the leucocyte was removed from its natural conditions in the animal body the bacteria sometimes apparently multiplied within the cell and finally burst and destroyed it. The phagocytic cells of the omentum evidently play an important part in pest immunity. In guinea pigs numbered 2153 and 2757 very much the same conditions were observed as in number 2152. The cells of the omentum perhaps contained not so many bacteria and there were a few leucocytes in the abdominal cavity which contained a few ingested bacilli. In number 2757 there was not as much exudate present as in number 2152. The local reaction about the point of inoculation was also more marked in guinea pig number 2757, there being a hæmorrhagic area about 2 cubic centimeters in diameter about the point of inoculation. In guinea pig number 2758 there was a very marked local reaction about the point of inoculation with extensive hæmorrhages in the tissues. In this animal there was practically no fluid in the abdominal cavity although there were some flakes and masses of fibrin containing a fair number of

SERIES 63—Continued.

leucocytes and there were only a few bacteria and a moderate number of leucocytes which contained bacteria in the abdominal cavity. These free organisms, however, took a distinct bipolar stain and some of them appeared capsulated. Films made by rubbing portions of the omentum upon glass slides and staining the preparations showed numerous polymorphonuclear leucocytes and large mononuclear phagocytes. A number of these contained bacteria, but not nearly so many cells had ingested bacteria as in the case of guinea pigs numbered 2152, 2153, and 2157, neither was the number of bacteria in the cells so great. In the abdominal exudate of these animals and in the omentum there was a distinct reduction in the number of the bacteria inoculated and it would appear that the organisms had invaded further into the tissues and to other organs such as the spleen and even the blood. The slight evidence of phagocytosis observed in this animal would appear to be due to the fact that the bacteria had not been acted upon by immune serum.

SERIES 64.—*Relating to the mechanism of the action of plague immune serum.*

Guinea pig number 2852: One agar slant culture of "Pest Virulent" from guinea pig number 2764, second transplant, was suspended in 1 cubic centimeter of saline solution. This suspension together with 1 cubic centimeter of pest immune serum was injected into the abdominal cavity of the animal. Microscopic examination of drops of fluid from the abdominal cavity after twenty minutes showed almost complete disappearence of the bacteria. There were numerous large mononuclear and polymorphonuclear cells present, none containing bacteria. After fifty minutes there was still no evidence of bacteria in the exudate, although the leucocytes were more numerous. The animal was killed one and one-half hours after inoculation. The omentum and posterior abdominal walls showed enormous numbers of bacteria, a large number of which had been ingested by leucocytes.

Guinea pig number 2853: One agar slant culture of "Pest Virulent" from guinea pig number 2852, second transplant, was suspended in 2 cubic centimeters saline solution. The animal was inoculated intraperitoneally with this suspension. Microscopic examination of drops of fluid examined from the abdominal cavity after twenty minutes showed numerous bacteria, many in groups. Comparatively few leucocytes were present. After fifty minutes apparently not much change had taken place. There were still many bacteria in the exudate and a few cells containing organisms. The animal was killed after one and one-half hours. A few leucocytes were found in the abdominal exudate; a number of these contained bacteria. In smears from the omentum enormous numbers of bacteria were found. There was little evidence of phagocytosis.

Guinea pig number 2593, *immune guinea pig:* Inoculated intraperitoneally with 1 agar slant culture of "Pest Virulent" from guinea pig number 2764, second generation, suspended in 2 cubic centimeters saline solution. Drops of fluid taken from the abdominal cavity after twenty minutes showed a few cells and a few free bacteria. After fifty minutes, numbers of large mononuclear cells were visible in the exudate, many of these were packed with bacteria, others contained bacteria lying about them. These organisms did not stain as deeply as normal bacteria. The animal was killed one and one-half hours after inoculation. An extensive phagocytosis of the bacteria by the cells of the omentum was found to have occurred. But there were also large numbers of bacteria which were not ingested.

Guinea pig number 2854: One-half agar slant culture "Pest Virulent" (from guinea pig number 2764, second transplant) was suspended in 0.5 cubic centimeter saline solution. To this suspension was added 0.5 cubic centimeter of pest

SERIES 64—Continued.

immune serum and the mixture injected into the abdominal cavity. Microscopic examination of drops of fluid after twenty minutes showed numerous bacteria with but few leucocytes. No evidence of phagocytosis was present. After sixteen hours the animal was killed. A small amount of sticky fluid was encountered in the abdominal cavity, containing numerous polymorphonuclear leucocytes, the great majority of which did not enclose bacteria. In the few which had ingested pest bacilli, the organisms appeared degenerated. Very few free bipolar staining organisms were present. In the omentum evidences of an extensive phagocytosis existed. However, there were still a number of free bacteria. Some of the bacteria had apparently invaded other organs of the body.

Guinea pig number 2855: One-half agar slant culture "Pest Virulent" (from guinea pig number 2764, second transplant) suspended in 1 cubic centimeter of saline solution was inoculated into the abdominal cavity. Microscopic examination of drops of fluid from the abdomen after twenty minutes showed numerous bacteria and comparatively few cells. After one hour a few large mononuclear cells containing bacteria were present. The number of bacteria was considerably diminished. The animal was killed after sixteen hours. Very few bacteria were found in the abdominal exudate. Upon the omentum and surface of the abdominal walls innumerable bacteria were observed. There were moderate numbers of leucocytes present, some of which contained organisms. There were also a number of free bacteria which took the bipolar stain and appeared encapsulated.

SERIES 65.—*Relating to the mechanism of the action of plague immune sera.*

Guinea pigs numbered 2751, 2860, 2862, 2864, 2867, 3050, and 3059 were all inoculated intraperitoneally with 1 cubic centimeter of pest immune serum, mixed with 1 cubic centimeter of a suspension of pest bacilli of the several strains "Pest Virulent," "Maassen Alt," and "Pest Avirulent." Guinea pigs numbered 2863, 2868, 3051, and 3060 were employed for controls and were inoculated with a suspension of the bacteria to which was added 1 cubic centimeter of saline solution in place of immune serum. Drops of fluid from the abdominal cavities of the animals were examined from time to time, from five minutes to one hour after the inoculation, the animals also being killed at various periods after the inoculation. These examinations demonstrated that the "Avirulent" strains of pest bacilli do to a certain extent become swollen and undergo degeneration under the influence of plague immune serum, outside of the phagocytes.

However, only a portion of the bacteria inoculated showed these changes, and a typical Pfeiffer's phenomenon with granular destruction of the bacilli does not occur, the degeneration manifesting itself by swelling of the bacilli and their poor and irregular staining reactions. The experiments on these animals also demonstrated the other phenomenon of phagocytosis, etc., encountered in the experiments described in the previous series.

The individual protocols are not further detailed here, the reults of the experiments having been fully described in general in the text. (See p. 262.)

XI. THE ANTI-INFECTIOUS POWER OF PLAGUE SERUM.

Since the report of Yersin, Calmette and Borrel in 1895,[88] practically all observers have recognized what may be termed the anti-infectious action of plague immune serum. The disputed point has been rather in regard to the extent of its curative value. As has already been mentioned, it was considered advisable to investigate to what amount these anti-infectious substances became developed in the blood of animals which had been immunized to plague infection by vaccination, and in others immunized in the same manner and the immunity of which to plague infection had in addition been tested, in order to ascertain whether or not these substances could be demonstrated in the blood of such animals in sufficient quantities for the method of their detection to be used as a test of the degree of immunity obtained in the serum of human beings vaccinated against plague. The method employed in investigating this question was as follows: A rat was inoculated intraperitoneally or subcutaneously with the serum to be tested while at the same time the animal was infected by thrusting beneath the skin near the tail a syringe needle which had been dipped in a suspension of the virulent pest organism. Numerous control animals were inoculated in all the series. Rats were used rather than mice in these experiments, since a much more accurate determination of the value of plague serum can be made with these animals than with mice. However, even with rats there is considerable individual variation in regard to their reaction to plague immune serum. Sometimes, after infection, one rat will be saved by a dose of serum, whereas another one of the same size and which has received the same amount of it will succumb. For this reason it is difficult and sometimes impossible accurately to determine the dose of serum which is just sufficient to save the animals. The method used in testing the protection obtained by the rats from the serum inoculations was somewhat more severe than that which has been used by other observers, the infecting needle employed in my experiments being larger; however, in spite of this, it is recognized that the values of the immune sera which I have experimented with were not high; a fact which may be seen from a study of the tables.

The details of the experiments are given in Series 8, 10, 13, 14, 15, 19, 20, 22, 27, 45, 53, and 57 (pp. 274 to 288).

[88] *Ann. d. l'inst. Pasteur* (1895), **9**, 589.

We learn from a study of these experiments that the anti-infectious substances also become developed very slowly and in very small quantities in animals immunized against pest. While rabbits which have been given a single, small, intravenous inoculation of either living or killed cholera or typhoid bacilli will develop a serum which in high dilutions is protective for guinea pigs against multiple lethal doses of these organisms, on the other hand, rabbits which have been intravenously inoculated with large amounts of killed virulent or with living, attenuated plague cultures, yield sera which, when tested on rats show apparently no protective power whatever against plague infection. Likewise, as large an amount as 5 cubic centimeters of a serum obtained from a rabbit previously inoculated intravenously with 20 milligrams of artificial plague aggressin proved to possess no anti-infectious power when tested on rats. Monkeys which had been immunized against pest by vaccination, or otherwise by inoculation, and which had been shown to be thoroughly immune by the subcutaneous inoculation of multiple lethal doses of the virulent pest strain, furnished sera which also showed no traces of anti-infectious power when tested on rats. Only in the case of one monkey (number 1357) could a slight anti-infectious power be noted and this animal had received repeated, increasing doses of virulent pest bacilli until it resisted the injection of 1½ agar slant cultures of the virulent pest strain. (See Series 57, p. 288.) This series of experiments is particularly important because it illustrates that the negative results in the demonstration of an anti-infectious action obtained with the serum of monkeys less highly immunized against pest, could not be ascribed to the lack of a suitable complement to complete the reaction in the body of the rat.

The sera of thirty-three human beings who had been vaccinated against pest by the inoculation of attenuated cultures were also tested, but in no instance did they show any demonstrable anti-infectious value. However, since animals which had proved themselves, thoroughly immune to pest infection also furnished sera which conferred no greater protection, it would not be reasonable to expect that the human sera would reach a higher power; moreover, the serum of a convalescent plague patient collected five days after the symptoms of the disease had subsided, also showed no anti-infectious value. Only in the case of horse's serum, where the animal had finally been inoculated with repeated, large doses of living pest bacilli, could any marked anti-infectious action be demonstrated and indeed with some of these sera it required as much as 1 cubic centimeter to save the rat from fatal pest infection. Therefore, it is unnecessary to emphasize further that the absence of the anti-infectious substances against pest in sufficient quantities to be demonstrated in a serum can not necessarily be regarded as an evidence of the absence of

even a considerable immunity against this disease in the individual in question, since in animals and human beings known to be thoroughly immune to pest infection it has also not been possible to demonstrate these protective substances.

EXPERIMENTS IN RATS DEMONSTRATING THE ANTI-INFECTIOUS POWER OF PLAGUE IMMUNE SERA.

SERIES: 8.—*Experiments with the species "Mus decumanus."*

One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters saline solution. A 5 cubic centimeter needle was dipped in the suspension and thrust under the skin near the root of the tail and then withdrawn. Animals numbered 1252, 1254, and 1256 received pest immune serum intravenously just before being stuck with the infected needle.

Animal No.	Serum.	Result.	Autopsy and remarks.
1249		Dead after 4 days.	No pest bacilli found in smears from heart's blood. Innumerable from region near point of inoculation. Bubonic abscess.
1250		Dead after 5½ days.	Few pest bacilli in heart's blood and in tissues near point of inoculation. Quite numerous in smears from spleen.
1251		Dead after 3½ days.	Numerous pest bacilli in heart's blood.
1252	1.25 cc. antipest serum intraperitoneally.	Alive	
1253		Dead after 3½ days.	Fair numbers of pest bacilli in heart's blood.
1254	1 cc. antipest serum intraperitoneally.	Alive	Animal very sick for 7 days after inoculation.
1255		Dead after 3½ days.	Pest septicæmia. Fair numbers of pest bacilli in heart's blood.
1256	1 cc. antipest serum intraperitoneally.	Alive	
1257		Dead after 3 days.	Smears and cultures from heart and spleen show few pest bacilli. On examining the tissues near the point of inoculation innumerable pest bacilli are found. Animal evidently died of pest intoxication.
1258		Dead after 7 days.	Bubo of the right inguinal gland in which pest bacilli are present. No organisms found in smears from the heart's blood.
1259		Dead after 5 days.	Countless pest bacilli in smears from the tissues near point of inoculation. None seen in smears from the heart's blood.
1260		Dead after 2 days.	No autopsy.

SERIES 10.—*Experiments with rats.*

One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters of saline solution. A 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Animals numbered 1265, 1267, 1268, and 1273 received intraperitoneally, just before being stuck with the infected needle, either human serum from cases previously vaccinated against plague, or horses' pest immune serum.

Animal No.	Serum.	Result.	Autopsy and remarks.
1264		Dead after 3½ days.	Few pest bacilli in heart's blood.
1265	3 cc. human serum case III.	Dead after 6½ days.	Numerous pest bacilli in smears from spleen and liver.
1266		Dead after 5 days.	Left inguinal bubo. Smears from it show numerous pest bacilli and a few cocci.
1267	2 cc. serum mixture human cases VI and VIII.	do	No organisms found in smears from blood, spleen, near point of inoculation, or right axillary gland. Cultures from the heart and spleen developed a few colonies of pest bacilli.
1268	3 cc. human serum case V.	Dead after 3½ days.	No bacilli found in the heart's blood. Countless pest bacilli in smears made from tissues near point of inoculation. Many bacilli swollen.
1269		do	Very few pest bacilli in smears from heart's blood. Much more numerous in smears from the flank.
1270		Dead after 5 days.	Cover slips from spleen show numerous pest bacilli.
1271		Dead after 3½ days.	Pest septicæmia. Fair numbers of pest bacilli in smears from heart's blood.
1272		do	Fair numbers of pest bacilli in smears from heart's blood.
1273	2.5 cc. antipest serum	Dead after 11 days.	Pest septicæmia. Fair numbers of pest bacilli in smears from spleen. Inguinal buboes with countless pest bacilli.
1274		Dead after 3 days.	Few pest bacilli in smears from heart's blood.
1275		Dead after 22 days.	Chronic pest. Buboes about point of inoculation contained pest bacilli. Numerous organisms in smears from spleen.

SERIES 13.—*Experiments with rats.*

One 48-hour agar slant culture "Pest Virulent" (from monkey number 1282, first transplant) was suspended in 5 cubic centimeters bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and then thrust beneath the skin of each animal near the root of the tail. Rats numbered 1308, 1310, 1312, 1314, 1316, 1318, and 1320 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases or from animals which had previously been vaccinated or otherwise inoculated against plague.

SERIES 13—Continued.

Animal No.	Serum intraperineally.	How inoculated.	Result.	Autopsy and remarks.
1307	----------	Stuck with 5 cc. syringe needle.	Dead after 3 days.	Numerous pest bacilli in the spleen.
1308	2 cc. serum from case 10.	-----do -------	-----do ------	Fair numbers of pest bacilli in smears from spleen.
1309	----------	-----do -------	Dead after 5 days.	Innumerable pest bacilli in smears from spleen.
1310	2 cc. serum from case 12.	-----do -------	Dead after 3 days.	Smears from spleen show a few bacilli. Organisms very numerous near point of inoculation.
1311	----------	-----do -------	-----do ------	Few pest bacilli in smears from spleen.
1312	2 cc. of serum from animal 1965.*	-----do -------	Dead after 2½ days.	Pest septicæmia. In smears from spleen numerous pest bacilli.
1313	----------	-----do -------	-----do ------	Numerous pest bacilli in smears from spleen.
1314	2 cc. serum from animal No. No. 1960.†	-----do -------	Dead after 3 days.	Numerous pest bacilli in smears from spleen. Many within leucocytes.
1315	----------	-----do -------	Dead after 1½ days.	Few pest bacilli in tissues about point of inoculation.
1316	2 cc. serum from animal No. 1960.	-----do -------	Dead after 3 days.	Numerous pest bacilli and post-mortem invading bacilli in smears from spleen.
1317	----------	-----do -------	-----do ------	Pest septicæmia. Innumerable pest bacilli in smears from the spleen.
1318	2 cc. serum from animal No. 1960.	-----do -------	-----do ------	Many post-mortem invading bacilli and a few pest bacilli in smears from the spleen.
1319	----------	-----do -------	Dead after 8 days.	Fair number of pest bacilli in smears from spleen, also a number of large post-mortem invading bacilli present.
1320	2 cc. from animal No. 1965.	-----do -------	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.
1321	----------	-----do -------	Dead after 15 days.	A few pest bacilli in smears from the spleen. None found near point of inoculation.

*1965 had been previously inoculated intravenously with 5 cubic centimeters Haffkine's prophylactic.

†1960 had been previously inoculated intravenously with one 48-hour culture "Pest Avirulent."

SERIES 14.—*Experiments with rats.*

A 3-day agar culture of "Pest Virulent" (first transplant) was suspended in 5 cubic centimeters of bouillon. A 5 cubic centimeter needle was dipped in this suspension and rats numbered 1330 to 1341 inoculated by thrusting the needle subcutaneously near the root of the tail. Rats numbered 1330 to 1337 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases which had previously been vaccinated against plague.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and Remarks.
1330	4 cc. from case 11.	Stuck with 5 cc. syringe needle.	Dead after 3 days.	Fair number of pest bacilli in smears from spleen. Abdominal cavity appears normal.
1331	5 cc. from case 12.	do	do	Only one pest bacillus found in a smear from the spleen. Countless numbers near the point of inoculation. Œdema, hæmorrhagic infiltration, etc., about this area. Abdominal cavity normal in appearance.
1332	5 cc. from case 9.	do	Dead after 5 days.	Very numerous pest bacilli in smears from the spleen.
1333	do	do	Dead after 3 days.	Innumerable pest bacilli in smears from the spleen. Abdominal cavity normal.
1334	2.5 cc. from case 17.	do	do	Innumerable pest bacilli in smears from spleen.
1335	do	do	Dead after 1½ days.	Abdominal cavity appears normal. No serum present. Fair numbers of pest bacilli in smears made from tissues near point of inoculation.
1336	2.5 cc. mixture from cases 18 and 10.	do	Dead after 3 days.	Fair number of pest bacilli in smears from spleen.
1337	do	do	Dead after 4 days.	Few bacilli in smears from spleen. Innumerable about point of inoculation. Infiltration and hæmorrhage about this area.
1338		do	Dead after 3 days.	Fair number of pest bacilli in smears from spleen.
1339		do	Dead after 4 days.	Very numerous pest bacilli in smears from spleen.
1340		do	Dead after 1½ days.	No organisms in smears from spleen. Fair numbers near point of inoculation.
1341		do	do	Fair numbers of pest bacilli about point of inoculation.

SERIES 15.—*Experiments with rats.*

One 48-hour agar culture "Pest Virulent" (guinea pig number 1296) suspended in 5 cubic centimeters bouillon; a 5 cubic centimter needle was dipped in this suspension and then thrust beneath the skin near the root of the tail of each animal. All the rats except the two numbered 1348 and 1353 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases or animals previously vaccinated against plague.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1342	5 cc. of serum from case 14.	Stuck with 5 cc. needle.	Dead after 4 days.	Fair number of pest bacilli in smears from spleen.
1343	2 cc. of serum from case 14.	do	do	Do.
1344	2.5 cc. serum from case 15.	do	Dead after 2 days.	Numerous pest bacilli and post-mortem invading bacilli in smears from spleen.
1345	do	do	Dead after 5 days.	Numerous pest bacilli in smears from spleen.
1346	5 cc. of serum from animal No. 1965 *	do	Dead after 3 days.	No bacilli found in smears from spleen. Innumerable from tissues near point of inoculation.
1347	do	do	do	Very few bacilli found in smears from spleen. Innumerable near point of inoculation.
1348		do	do	Innumerable pest bacilli in smears from the spleen.
1349	5 cc. serum from animal No. 1967. †	do	Dead after 4 days.	A fair number of pest bacilli in smears from spleen.
1350	do	do	Dead after 2 days.	No pest bacilli seen in smears from spleen. A few post-mortem invading bacilli present.
1351	2.5 cc. serum from animal No. 1967.	do	Dead after 3 days.	A few pest bacilli in smears from spleen.
1352	do	do	Dead after 4 days.	Fair number of pest bacilli in smears from spleen. Numerous post-mortem invading bacilli.
1353		do	Dead after 3 days.	Few pest bacilli in smears from spleen.

* Number 1965 had been previously inoculated intravenously with 5 cubic centimeters Haffkine's prophylactic.

† Number 1967 had been previously inoculated intravenously with 1 cubic centimeter autolyzed product digested from 20 milligrams of the strain "Pest Virulent."

SERIES 19.—*Experiments with rats.*

November 9, one 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1356, first transplant) was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and each animal inoculated by thrusting the needle beneath the skin near the root of the tail. Animals numbered 1386 to 1407 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases previously vaccinated against plague. Rat number 1412 received 0.5 cubic centimeters of an antipestic immune serum.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1386	4.25 c c. from case 19.	Stuck with infected needle.	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1387	——do——	——do——	——do——	Do.
1388	4 cc. from case 20.	——do——	——do——	Do.
1389	——do——	——do——	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1390	2 cc. from case 21.	——do——	——do——	Numerous pest bacilli in smears from spleen. Intestinal wall dark and hæmorrhagic in places.
1391	3.5 cc. from case 23.	——do——	——do——	Innumerable pest bacilli in smears from spleen.
1392	——do——	——do——	——do——	Intestine hæmorrhagic in places. Innumerable pest bacilli in smears from spleen.
1393	3 cc. from case 24.	——do——	——do——	Innumerable pest bacilli in smears from spleen.
1394	——do——	——do——	——do——	Very few pest bacilli in smears from the spleen. Innumerable near the point of inoculation.
1395	3 cc. from case 25.	——do——	——do——	A few pest bacilli in smears from the spleen.
1396	4 cc. from case 26.	——do——	——do——	Numerous pest bacilli in smears from the spleen.
1397	——do——	——do——	Dead after 3 days.	A very few typical pest bacilli in smears from spleen.
1398	4.5 cc. from case 27.	——do——	——do——	A fair number of pest bacilli in smears from the spleen, appearing in clumps.
1399	——do——	——do——	Dead after 4 days.	Numerous pest and post-mortem invading bacilli in smears from the spleen.
1400	5 cc. from case 28.	——do——	Dead after 8 days.	No bacilli found in smears from the spleen or near point of inoculation.

SERIES 19—Continued.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1401	5 cc. from case 28.	Stuck with infected needle.	Dead after 7 days.	No pest bacilli found in smears from the spleen or in tissues near the point of inoculation. Spleen small. No infiltration about point of inoculation. It is not clear that this rat died of pest infection.
1402	4 cc. from case 29.	do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1403	do	do	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1404	2.5 cc. from case 31.	do	do	Very few pest bacilli in smears from the spleen. Innumerable in smears from tissues near point of inoculation.
1405	do	do	Dead day of inoculation.	
1406	3.25 cc. from case 33.	do	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1407	do	do	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1408		do	Dead after 2 days.	A few pest bacilli in smears from spleen.
1409		do	Dead after 4 days.	A fair number of typical pest bacilli in smears from spleen.
1410		do	Dead after 6 days.	Very numerous pest bacilli in smears from spleen.
1411		do	Dead after 4 days.	Do.
1412	0.5 cc. antipestic serum.	do	do	Numerous pest bacilli in smears from the spleen. Many swollen. A few near the point of inoculation.
1413		do	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.

It might be argued that not a sufficient number of animals were inoculated in the different series with each serum for the results to be conclusive, since it is well known that in a series of rats inoculated with a pest immune serum of high value, a certain number of animals are not protected and always die of pest infection after inoculation with a virulent organism, and indeed, the single animal of this series inoculated with the horse's pest immune serum succumbed to pest. However, it seems probable that in such a long series of animals, if there were any the blood of which possessed marked anti-infectious power, the evidences of this fact would be presented by at least some of the animals. Only in the serum of case 28 is there any evidence whatever of an anti-infectious power and there is no further evidence of this nature in any of the other series of rats which have been inoculated with serum from human cases vaccinated against plague. (Compare with Series 22, p. 281 animals 1454 and 1455.)

SERIES 20.—*Experiments with rats.*

November 11, one 70-hour agar slant culture of "Pest Virulent" (from guinea pig number 1354, first transplant), was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Animals numbered 1423, 1425 and 1427, 1429 and 1431 and 1433 received intraperitoneally 1 to 2 cubic centimeters of an antipest immune serum just prior to being stuck with the infected needle.

Animal No.	Amount of serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1423	2 cc. antipest serum.	Stuck with 5 cc. syringe needle.	Dead after half hour.	Carbolic acid poisoning.
1414	------	---do---	Dead after 4 days.	Few bacilli in smears from spleen. Innumerable near point of inoculation.
1425	2 cc. antipest serum.	---do---	Dead after 11 days.	No organisms found in smears near point of inoculation. In cultures from spleen no pest bacilli.
1426	------	---do---	Dead after 4 days.	Innumerable pest bacilli in smears from tissues near point of inoculation.
1427	2 cc. antipest serum.	---do---	Alive	
1428	------	---do---	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1429	2 cc. antipest serum.	---do---	Dead after ½ hour.	Carbolic acid poisoning
1430	------	---do---	Dead after 5 days.	Very numerous pest bacilli in smears from spleen.
1431	1 cc. antipest serum.	---do---	Alive	
1432	------	---do---	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.
1433	0.5 cc. antipest serum.	---do---	Dead after 1 day.	Probably carbolic poisoning.
1434	------	---do---	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.

SERIES 22.—*Experiments with rats.*

On November 17, one 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1383, second transplant) was suspended in 5 cubic centimeters bouillon, a 5 cubic centimeter needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Rats numbered 1453 to 1463 inclusive received intraperitoneally, just prior to being stuck with the infected needle, serum of monkeys which had been previously inoculated and subsequently had been shown to be entirely immune to large doses of the strain "Pest Virulent."

SERIES 22—Continued.

Animal No.	Serum intraperitoneally.	How infected.	Result.	Autopsy and remarks.
1453	2 cc. serum from monkey No. 1285.*	Stuck with infected needle.	Dead after 4 days.	Very few pest bacilli in smears from point of inoculation.
1454	2 cc. serum from monkey No. 1278.†	do	Dead after 12 days.	Few pest bacilli in smears from spleen. None found at point of inoculation.
1455	do	do	Dead after 6 days.	Numerous pest bacilli in smears from spleen.
1456	do	do	do	Numerous pest bacilli near point of inoculation. None seen in smears from the spleen.
1457	do	do	Dead after 4 days.	Very few bacilli in smears from spleen. No local lesion apparent.
1458	0.75 cc. serum from monkey No. 1278.	do	Dead after 5 days.	Few bacilli in smears from spleen. Spleen greatly swollen.
1459	2 cc. serum from monkey No. 1231.‡	do	Dead after 6 days.	No pest bacilli found in smears from the spleen. Much necrosis and œdema of tissue near point of inoculation.
1460	do	do	Dead after 5 days.	Very numerous pest bacilli in smears from the spleen.
1461	do	do	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1462	do	do	Dead after 1 day.	
1463	do	do	Dead after 5 days.	Very numerous pest bacilli in smears from spleen.
1464		do	Dead after 4 days.	Do.
1465		do	do	Numerous pest bacilli in smears from spleen.
1466		do	Dead after 2 days.	Few pest bacilli near the local lesion.
1467		do	Dead after 3 days.	Innumerable pest bacilli in smears from spleen.
1468		do	Dead after 5 days.	A few pest bacilli in smears from the spleen.
1469		do	Dead after 4 days.	Fairly numerous pest bacilli in smears from spleen.
1470		do	Dead after 3 days.	Fair number pest bacilli in smears from spleen.

*Monkey number 1285 was inoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle which had been dipped in a bouillon suspension of an agar slant of "Pest Virulent." It suffered a mild pest infection and on October 25 was reinoculated with 2 oesen of the strain "Pest Virulent." It survived this inoculation and on November 16 was killed by bleeding and the value of its serum tested as above. Its agglutinative power tested November 17 was found to be entirely negative even in dilutions of 1 or 2.

†Monkey number 1278 was vaccinated October 19 with a culture of "Pest Avirulent." On October 30 it was reinoculated with 2 oesen "Pest Virulent," and proved to be immune. It was killed November 16 by bleeding and the anti-infectious value of its serum tested as above. Its agglutinative power tested November 17 was entirely negative in dilutions as low as 1 to 2 or 1 to 4 after four hours.

‡Monkey number 1231 was inoculated on October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle that had previously been dipped in a suspension of an agar slant of "Pest Virulent." It suffered a mild pest infection and on October 25 was inoculated with 2 oesen of "Pest Virulent" which it survived. On November 16 it was killed by bleeding and the anti-infectious power of its serum tested as above. Its agglutinative value with the strain "Pest Virulent" was tested on November 17 and found to be entirely negative in dilutions of 1 to 2 and 1 to 4 after four hours.

SERIES 27.—*Experiments with rats.*

One 48-hour agar slant culture of "Pest Virulent" from guinea pig numbered 1668, first transplant), was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Rats numbered 1677 to 1686 received intraperitoneally pest immune serum just before being stuck with the infected needle.

Animal No.	Inoculated intraperitoneally with—	How infected.	Result.	Autopsy and remarks.
1677	1 cc. pest immune serum.	Stuck with infected needle.	Dead after 7 days.	Very numerous pest bacilli and post-mortem organisms in smears from spleen.
1678	do	do	Alive and well.	
1679	do	do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1680	do	do	Dead January 31, after 12 days.	Do.
1681	do	do	Alive and well.	
1682	0.5 cc. pest immune serum.	do	Dead January 22, after 3 days.	Innumerable typical pest bacilli from point of inoculation. Few if any organisms in smears from the spleen.
1683	do	do	Dead after 8 days.	Fair numbers of typical pest bacilli in smears from the spleen.
1684	do	do	Dead after 4 days.	Do.
1685	do	do	Dead after 6 days.	Innumerable pest bacilli in smears from spleen.
1686	do	do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1687		do	Dead after 6 days.	Very numerous pest bacilli in smears from spleen.
1688		do	Dead after 3 days.	No organisms found in smears from spleen. Innumerable pest bacilli near point of inoculation.
1689		do	do	Innumerable pest bacilli in smears from spleen.
1690		do	do	Fair numbers of typical pest bacilli in smears from spleen.
1691		do	Dead after 17 days.	Typical pest bacilli from the local lesion. Fair numbers of typical organisms in smears from the spleen.
1692		do	Dead after 4 days.	Numerous typical pest bacilli in smears from spleen.
1693		do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1694		do	do	Very numerous typical pest bacilli in smears from the spleen.
1695		do	Dead after 4 days.	Innumerable pest bacilli in smears from the spleen.
1696		do	do	Do.

SERIES 45.—*Experiments with white rats.*

October 26, one 48-hour agar culture of "Pest Virulent" (from guinea pig number 2583, second transplant) was suspended in 5 cubic centimeters of bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin of the animal near the base of the tail. Ten of the rats received intraperitoneally 1 cubic centimeter of pest immune serum at the time of being stuck with the infected needle, and ten received the same amount of serum twenty-four hours after the infection. Three received, at the time of the infection, human serum from a case which had suffered from bubonic plague and recovered. The blood was collected five days after the symptoms of the disease had subsided and the serum separated and preserved in 0.5 per cent carbolic acid. The remaining 15 rats received no serum.

Animal No.	Serum intraperitoneally.	Infected October 26.	Result.	Autopsy and remarks.
2595	Oct. 26, 1 cc. pest immune serum.	Stuck with 5 cc. syringe needle dipped in suspension "Pest Virulent."	Dead Oct. 30, after 4 days.	No pest bacilli in smears from spleen. Innumerable about point of inoculation.
2596	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 31, after 5 days.	Very few bacilli in smears from spleen. Numerous in tissues near point of inoculation.
2597		do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli near point of inoculation.
2598	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 3, after 8 days.	Very few bacilli in smears from spleen. Hæmorrhagic area at point of inoculation, containing numerous bacilli.
2599	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 30, after 4 days.	Fair number of pest bacilli in smears from the spleen.
2600		do	do	Very numerous pest bacilli in smears from spleen.
2601	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2602	Oct. 27, 1 cc. pest immune serum.	do	do	
2603		do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2604	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2605	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 31, after 5 days.	Fair number of pest bacilli in smears from spleen.
2606		do	Dead Oct. 29, after 3 days.	Do.
2607	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 1, after 6 days.	Few pest bacilli in smears from spleen. More numerous near point of inoculation.
2608	Oct. 27, 1 cc. pest immune serum.	do	Alive and well.	
2609		do	do	
2610	Oct. 26, 1 cc. pest immune serum.	do	do	

SERIES 45—Continued.

Animal No.	Serum intraperitoneally.	Infected October 26.	Result.	Autopsy and remarks.
2611	Oct. 27, 1 cc. pest immune serum.	Stuck with 5 cc. syringe-needle dipped in suspension "Pest Virulent."	Alive and well.	
2612	do	do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli in smears from point of inoculation.
2613	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2614	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 29, after 3 days.	Fair number of pest bacilli in smears from spleen.
2615	do	do	do	Moderate numbers of pest bacilli in smears near point of inoculation.
2616	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 2, after 7 days.	Few pest bacilli in smears from the spleen. About point of inoculation a red spot about 3 mm. in diameter. Smears from this area show numerous pest bacilli.
2617	Oct. 27, 1 cc. pest immune serum.	do	Alive and well.	
2618	do	do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2619	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2620	Oct. 27, 1 cc. pest immune serum.	do	Dead Nov. 2, after 7 days.	Few swollen pest bacilli in smears from spleen. Fairly numerous about point of inoculation, where cocci are also present.
2621	do	do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2622	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2623	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 30, after 4 days.	Few pest bacilli in smears from spleen. Numerous from point of inoculation.
2624	do	do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli in smears from spleen.
2625	Oct. 26, 2 cc. human serum.	do	do	Very numerous pest bacilli in smears from spleen.
2626	Oct. 26, 1 cc. human serum.	do	do	Fair number of pest bacilli in smears from spleen.
2627	do	do	do	Innumerable pest bacilli in smears from spleen.
2628	do	do	Dead Oct. 30, after 4 days.	Few pest bacilli in smears from spleen.
2629	do	do	Dead Oct. 29, after 3 days.	Numerous pest bacilli in smears from spleen.
2630	do	do	Dead Oct. 31, after 5 days.	Do.
2631	Oct 26, 1 cc. human serum	do	Dead Oct. 29, after 3 days.	Do.
2632	do	do	do	Do.

SERIES 53.—*Experiments with white rats.*

November 6, one 3-day culture of "Pest Virulent" from guinea pig number 2594, second transplant, was suspended in 5 cubic centimeters bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and each rat inoculated by thrusting the needle beneath the skin near the root of the tail. Ten of the rats were given subcutaneously 1 cubic centimeter of a pest immune serum at the time of the infection with the needle. Ten others were given the same amount of serum twenty-four hours after the infection, and those which remained alive of the third ten (nine) were inoculated with 1 cubic centimeter of the serum forty-eight hours after the infection. Ten others were given subcutaneously, at the same time as the others, 1 cubic centimeter of this pest immune serum, which however had been treated previously with the virulent pest strain as described on page 261.

Animal No.	Infected November 6.	Immune serum, 1 cc. subcutaneously on—	Result.	Autopsy and remarks.
2768	Stuck with 5 cc. syringe needle.	Nov. 6	Alive and well.	
2769	do	Nov. 7	Dead Nov. 13, after 7 days.	No pest bacilli found in smears from spleen. One or two swollen organisms found after prolonged search in smears from tissues near point of inoculation.
2770	do	Nov. 8	Alive and well.	
2771	do	Nov. 6	do	
2772	do	Nov. 7	Dead Nov. 9, after 3 days.	Fairly numerous pest bacilli in smears from spleen.
2773	do	Nov. 8	Dead Nov. 11, after 5 days.	No bacilli in smears from spleen. About point of inoculation hæmorrhagic area in which numerous pest bacilli are present.
2774	do	Nov. 6	Dead Nov. 14, after 8 days.	Smears from spleen show one or two swollen bacilli. Smears from point of inoculation show no organisms. No other evidences of pest infection.
2775	do	Nov. 7	Dead Nov. 11, after 5 days.	Numerous post-mortem bacilli and a few pest bacilli in smears from the spleen.
2776	do	Nov. 8	Dead Nov. 9, after 3 days.	Very numerous pest bacilli in smears from spleen.
2777	do	Nov. 6	Alive and well.	
2778	do	Nov. 7	Dead Nov. 11, after 5 days.	No bacilli in smears from spleen. Very numerous fat point of inoculation.
2779	do	Nov. 8	Dead Nov. 12, after 6 days.	Fairly numerous pest bacilli about point of inoculation. None found in smears from spleen.
2780	do	Nov. 6	Alive and well.	
2781	do	Nov. 7	do	
2782	do	Nov. 8	Dead Nov. 11, after 5 days.	Numerous pest bacilli in smears from the spleen.

SERIES 53.—Continued.

Animal No.	Infected November 6.	Immune serum, 1 cc. subcutaneously on—	Result.	Autopsy and remarks.
2783	Stuck with 5 cc. syringe needle.	Nov. 6	Alive and well.	
2784	do	Nov. 7	do	
2785	do	Nov. 8	do	
2786	do	Nov. 6	do	
2787	do	Nov. 7	do	
2788	do	Nov. 8	do	
2789	do	Nov. 6	do	
2790	do	Nov. 7	do	
2791	do	Nov. 8	Dead Nov. 9, after 3 days.	Very numerous pest bacilli in smears from spleen.
2792	do	Nov. 6	Alive and well.	
2793	do	Nov. 7	do	
2794	do		Dead Nov. 8, after 2 days.	Innumerable pest bacilli in smears from spleen.
2795	do	Nov. 6	Alive and well.	
2796	do	Nov. 7	do	
2797	do	Nov. 8	Dead Nov. 8, after ½ hour (serum).	Marked symptoms of carbolic poisoning after inoculation of serum. Animal died few minutes after injection. Numerous pest bacilli in smears from spleen.
		CONTROLS.		
2798	Stuck with 5 cc. syringe needle.		Dead Nov. 8, after 2 days.	Innumerable pest bacilli in smears from spleen.
2799	do		Dead Nov. 9, after 3 days.	Do.
2800	do		do	Pest and post-mortem bacilli in smears from spleen.
2801	do		Dead Nov. 10, after 4 days.	Fair number of pest bacilli in smears from spleen.
2802	do		Still alive	
2803	do		Dead Nov. 9, after 3 days.	Few bacilli in smears from spleen. Numerous at point of inoculation.
2804	do		do	Innumerable pest bacilli in smears from spleen.
2805	do		Dead Nov. 10, after 4 days.	Numerous pest bacilli in smears from spleen.
2806	do		do	Do.
2807	do		do	Do.

SERIES 53—Continued.

Animal No.	Infected November 6.	Immune serum after binding of ambo-ceptors.	Result.	Autopsy and remarks.
2810	Stuck with 5 cc. syringe needle.	Nov. 6, 1 cc.	Dead Nov. 10, after 9 days.	Pest bacilli in smears from spleen.
2811	do	do	Dead Nov. 11, after 5 days.	Do.
2812	do	do	do	Do.
2813	do	do	Alive and well.	
2814	do	do	Dead Nov. 13, after 7 days.	Do.
2815	do	do	Dead Nov. 10, after 4 days.	Do.
2816	do	do	Alive and well.	
2817	do	do	do	
2818	do	do	Dead Nov. 11, after 5 days.	Do.
2819	do	do	Dead Nov. 13, after 7 days.	Do.

SERIES 57.—*Experiments with rats.*

February 23, one 48-hour culture of "Pest Virulent" from guinea pig numbered 3079, second transplant, was suspended in 5 cubic centimeters bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and each rat inoculated by thrusting the needle beneath the skin near the root of the tail. Five of the rats were giving subcutaneously varying amounts of serum from a monkey (numbered 1357 *) highly immunized against plague by repeated inoculations in increasing doses of the strain "Pest Virulent." The other rats which served as controls were given no serum.

Animal No.	Serum intraperitoneally.	How infected.	Result.	Autopsy and remarks.
3087	3 cc. serum from monkey No. 1357.*	Stuck with 5 cc. syringe needle.	Alived and well	
3088	do	do	Dead after 14 days	Few pest bacilli in point of inoculation.
3089	2 cc. of serum from monkey No. 1357.	do	Alive and well	
3090	do	do	Dead after 6 days	Few pest bacilli in smears from spleen.
3091	1 cc. serum from monkey No. 1357.	do	Dead after 4 days	Numerous pest bacilli in smears from spleen.
3095		do	do	Do.
3096		do	Dead after 3 days	Do.
3097		do	Dead after 4 days	Do.

*Monkey number 1357 was inoculated as follows: November 2 infected by sticking with a 5 cubic centimeter syringe needle dipped in a suspension of the strain "Pest Virulent". December 2, one-fifth agar slant culture of Pest "Virulent" subcutaneously. December 14, three-fifths of an agar slant, same strain, subcutaneously. January 10, one agar slant "Pest Virulent," subcutaneously. February 1, one and one-half agar slant cultures, "Pest Virulent" subcutaneously. Bled February 22. Twenty cubic centimeters; serum tested as above.

XII. CURATIVE VALUE OF PLAGUE SERUM.

The opinions of different observers are widely divergent in regard to the action of pest immune serum upon the clinical course of the disease in human beings. Amost all authors agree that in advanced cases of infection the serum is of no value. It is not my purpose here to enter into a complete discussion of this subject. The question has recently been reviewed during the present year (1906) by Dujardin-Beaumetz,[89] by Choksy,[90] and by Terni.[91] In 1905 Bannerman[92] reviewed the question of serum therapy in plague in India and the earlier work has been carefully summarized and considered by Dieudonné.[93] From a study of the cases treated and reported in these articles, together with the results obtained from experimentation with animals in the laboratory, there would appear to be little doubt of the value of the serum treatment of plague, provided that the serum is given early enough in the course of the disease. Up to the present time we have not been able to obtain a serum which shows any demonstrable antitoxic value by any known method of preparation, since indeed we have been unable to obtain a soluble pest toxin. The work of Markl upon this subject has already been considered on page 184 of this article.

Besredka's[94] studies upon the endotoxins of typhoid and pest bacilli may be mentioned in connection with the question, but they have not been elaborated further. Terni[95] thought that he was able to prepare an anti-serum which was especially active against the specific pest toxin, by the inoculation of the animal furnishing the serum with peritoneal exudates from guinea pigs dead of pest and with the serum from pest buboes, etc. (natural plague aggressin). Such a serum he regarded as being better than those sera obtained by the other known methods. However, I have shown (see p. 236) that the immunity obtained by the injection of natural plague aggressin is not of a different nature from that secured by the inoculation of living pest cultures and that, while inoculations of natural plague aggressin produce an immunity greater than that which can be obtained from those of artificial plague aggressin, the

[89] *Bull. d. l'inst. Pasteur.* (1906), **4**, 473.
[90] Report on the Treatment of Plague, Bombay (1906).
[91] *Ztschr. f. Hyg. u. Infectionskrankh.* Leipz. (1906), **54**, 386.
[92] *Scient. Mem. Med. Off. India* (1905), 20.
[93] *Handb. d. path. Mik.* (Kolle & Wassermann) (1904), **4**, 949.
[94] *Ann. d. l'inst. Pasteur* (1905), **19**, 477.
[95] *Loc. cit.*

inoculation of living cultures of pest bacilli gives rise to a higher immunity than either of the first two methods mentioned.

As I have already explained in discussing the subject of the mechanism of the action of plague immune serum, the serum exerts its immunizing effect primarily by binding through its amboceptors the receptors of the plague bacilli and after this change has taken place, phagocytosis of the microörganisms occurs. The success of the serum treatment in plague would appear to depend particularly upon the number of plague bacilli in the animal organism at the time of the inoculation of the serum; that is, upon the length of time the serum is injected after the infection has occurred. If the organism is already overwhelmed with bacteria at the time of the introduction of the serum, almost no favorable change will be noted in the course of the disease, because the serum is merely anti-infectious and is not anti-toxic. A study of the experiments recorded in Series 45 and 53 (pp. 284 and 286) confirms these views. In Series 45 we see that in ten rats inoculated with immune serum at the time of their infection with pest bacilli, 60 per cent survived and 40 per cent succumbed to the infection, while of ten rats which were inoculated with the serum twenty-four hours *after* the pest infection only 40 per cent survived and 60 per cent died. The experiments recorded in Series 53 are particularly important in this connection. Here the animals were inoculated with the serum in three series: One at the time of the infection, a second twenty-four hours following the infection, and the third forty-eight hours after the infection. The mortality in the first series was 10 per cent, in the second 40 per cent, and in the third 66.6 per cent.

It is true that there is nothing new in the idea that rodents may be protected against a fatal outcome of the pest infection by the injection of plague immune serum which has been introduced at varying periods of time after that of the infection. In fact, as long ago as 1895, Yersin, Calmette and Borrel[96] called attention to the fact that mice which had been infected with pest could be saved from death if an inoculation of 1.5 cubic centimeters of pest immune serum were to be given them as late as twelve hours after the time of the pest infection. Moreover, this method is still used in the Pasteur Institute and elsewhere (usually with rats), for the purpose of testing the curative value of the manufactured pest immune serum.

The German Plague Commission also found it possible to save monkeys which had previously been infected with pest by the inoculation of pest immune serum injected as late as from twelve to twenty-four hours after the time of the infection, and many other observers have recorded numerous similar successful experiments in which rats were employed. I therefore have not called attention to my experiments recorded in Series 51 with the sole object of again illustrating this action of plague immune serum;

[96] *Ann. d. l'inst. Pasteur* (1895), **9**, 591.

however, this series of experiments serves to illustrate particularly well the relations between the duration of the infection and results of the serum treatment.

For example, in the case of rat number 2797 which succumbed from carbolic acid poisoning one-half hour after the injection of the immune serum, an autopsy performed immediately after death showed that numerous pest bacilli already had invaded the spleen. This animal had been infected with pest on November 6 and the serum was inoculated two days later, November 8. Rats numbered 2794 and 2798, which were also infected on September 6, died on September 8 of pest infection before receiving serum. At autopsy the infection likewise was found to be well advanced in both animals and innumerable bacilli were present in each instance in the spleen.

It therefore seems fair to presume that, in a good proportion of the other rats inoculated on November 6 with the same sized dose, the infection had also already advanced to a somewhat similar stage, and therefore probably in many of these animals the pest bacilli had also already invaded the spleen. Nevertheless, 33.3 per cent of those animals which received the serum forty-eight hours after the time of infection survived. Therefore, these experiments demonstrate not only that animals may be saved by the injection of immune serum at a period subsequent to the incurrence of the pest infection, but that animals in which the disease is fairly well advanced may also be saved by the serum, the percentage of deaths varying directly with the length of the interval between pest infection and serum inoculation.

Choksy, who has a wide experience with the serum treatment of plague, states:

> Much depends upon the early and free use of the serum. In patients injected on the first day or within a few hours of the onset of the symptoms, one injection of 100 cubic centimeters followed by another after 6 to 8 hours and then if necessary by a third after a similar interval, would cut short the attack if the case be not pneumonic, malignant or septicæmic.

He also emphasizes the fact that the earlier the serum is used, the more efficacious will it be and that if good results are to be obtained from serum therapy the patient must be treated on the first day of the illness. He admits that the serum can not favorably influence all types of plague, or even the malignant forms of the bubonic type, but he shows that it is the only treatment capable of saving a large proportion in a certain class of patients. Therefore, it seems that in man there is a narrow limit beyond which the antiplague serum will not act, but that within this limit its use in man, as in animals, is efficacious.

Roux, Yersin, and Dujardin-Beaumetz all emphasize that in the serum treatment of plague, as in that of cholera, the injection of the serum must be given intravenously, on the ground that the absorption from

the cellular tissue is very slow and that by the intravenous inoculation the organism may be saturated with the antitoxin immediately and by a smaller dose than it could be if used subcutaneously. Dujardin-Beaumetz [97] further remarks that in cases where the pest bacilli appear early in the general circulation, there are additional advantages for the intravenous inoculation of serum. In my opinion in cases where plague bacilli are already circulating in the blood there is little hope of a successful result with the serum, either in man or animals. The treatment of cholera with intravenous inoculations of the "antitoxic cholera serum" of Brau and Denier so far has not been very successful, apparently because the serum does not evidence sufficient antitoxic action. In plague immune serum also, no matter by what method it is prepared, as has been demonstrated, we are not able to obtain any antitoxic action of value and so, for the same reason, we perhaps may not be justified in the hope of obtaining in plague any different results from the intravenous than from the subcutaneous inoculation of the serum, other than those which, as the French authors have suggested, would result from its more rapid absorption. By the intraperitoneal inoculation, as suggested by Dujardin-Beaumetz and others, a rapid absorption of the serum may also be secured. The cases of plague in which serum therapy will accomplish the best results are those where the infection is still confined to certain of the lymph channels. There is no doubt of the great prophylactic value of plague immune serum but, as was mentioned in Chapter IV, the passive immunity conferred by it disappears after a few days.

[97] *Loc. cit.*, p. 481.

XIII. VIRULENCE OF THE PLAGUE BACILLUS.

INCREASE OF VIRULENCE OF THE ORGANISM.

The behavior of the pest bacillus in relation to its virulence is very interesting and in many respects quite unusual, when it is compared with that of the majority of the microörganisms giving rise to our other common infectious diseases. The question obviously is also a very important one from an epidemiological standpoint and therefore will be discussed somewhat at length.

In 1895, Yersin, Calmette and Borrel [98] maintained that in a series of passages of the pest bacillus through a given species of animal, an increase of virulence ("a fixed virulence") was obtained for this species of animal only, and that by continued passages through a series of animals of one species, its virulence for other species became reduced. Dujardin-Beaumetz,[99] during the present year also speaks of a hyper-virulence of the pest bacillus obtained by passage through guinea pigs and rats. Hankin [100] found that the virulence of plague bacilli became reduced by passage through rats and that he was not able to kill more than three or four rats in a series, even though the culture in the beginning was fully virulent. On the other hand, he found that the virulence of the organism could be increased by passage from mouse to mouse.

Walton, [101] also observed that a virulent strain of pest became attenuated by successive passages from rat to rat. He used a suspension of a portion of the spleen of the dead animal for infecting the second one, and so on. He also was not able to infect more than three rats in a series by this method. Otto,[102] from numerous carefully performed experiments, concluded first that in the passage of pest bacilli from guinea pig to guinea pig without growth on artificial media, there resulted no dimunition in virulence of the organism either for the guinea pig or for the rabbit, rat or mouse, and second, that it did not appear to be possible by passing the organism successively through guinea pigs to obtain a substantial increase in the virulence of a culture of sufficient pathogenesis at the time of the beginning of the experiment, to cause the death of a guinea pig.

The Plague Commission in India under the direction of Martin and Lamb [103] have also very recently reported upon the effect upon its virulence of the passage of *Bacillus pestis* through series of rats, when the organism was given by subcutaneous inoculation without intermediate culture. Each rat in the series was infected with a suspension of the spleen of the animal which had died previously.

[98] *Ann. de l'Inst. Pasteur* (1895), **9**, 589.
[99] *Bull. de l'Inst. Pasteur* (1906), **4**, 473.
[100] Rept. of the Indian Plague Commission (1899), **2**, 22.
[101] Rept. of Indian Plague Commission (1900), **3**, 337.
[102] *Zeit. f. Hyg. u. Infectionskrankh.* Leipz. (1902), **41**, 380; (1904), **48**, 430.
[103] *Jour. de Hyg.* (1906), **6**, 496, 502.

It was found possible to carry on the series in this way as far as the twenty-sixth passage, when apparently the experiments were discontinued. The rats were all wild, Bombay rats and were not selected with reference to species. No evidence was obtained by these experiments that the virulence of the bacillus used for the passages underwent any alteration nor was any given by those in which cutaneous inoculation of a series of rats was attempted. Notwithstanding the failure of Otto and of the Indian Plague Commission perceptibly to increase the virulence of pest bacilli by repeated inoculation from guinea pig to guinea pig or from rat to rat, Dieudonné[104] states that pest cultures which have become weakly virulent may again be rendered more infectious by repeated passages through such animals as guinea pigs and rats. He also recommends the passage from the pneumonic lung of the infected animal to the healthy lung of the second animal, such as the rat or ape, and so on through a series as a means of increasing the virulence of this organism.

Balzaroff[105] also found that the virulence of a pest strain could be increased if it was passed successively for several generations from the pneumonic lung of an animal, by nasal infection, to the healthy lung of another one.

An organism which had not the power of killing animals by hypodermic inoculation was said to be restored to virulence by inoculating a guinea pig with it in the nostril and upon the death of this animal, inoculating by the same portal of entry a second one with a portion of the spleen of the first. After the third or fourth passage the organism was said again to have become virulent.

Marsh[106] claimed that if the organism was cultivated at certain temperatures in mixtures of carbonic acid gas and ordinary air, the bacillus increased in virulence and retained its vitality for a long time.

Albrecht and Gohn,[107] and Kolle and Martini[108] also found that by means of repeated animal passages, without growth on artificial media, a strain of the pest bacillus was rendered more virulent for the species of animal employed in the experiment, and also for other species which were markedly susceptible to pest. On the contrary, the transmission of the pest virus from rabbit to rabbit apparently resulted in a reduction of the virulence of the organism.

Kolle and Hetsch[109] also state that some strains of pest bacilli can be rendered more virulent by animal passage.

My experiments, which had the object of increasing the virulence of the pest bacillus, have been carried on in guinea pigs and monkeys and both virulent and avirulent strains of the organism have been used. However, no perceptible increase in virulence has been obtained for either rats, guinea pigs or monkeys. The strain "Pest Virulent," as has already

[104] *Handb. d. path. Mik.* (Kolle and Wassermann) (1903), **2**, 516.

[105] *Ann. d. l'inst. Pasteur* (1899), **13**, 385.

[106] Rep. of Indian Plague Commission (1898–99), **3**, 73; also (1898–99), **5**, App. III, 480.

[107] Loc. cit.

[108] *Deutsche med. Wchnschr.* (1902), **28**, 1.

[109] Die Exper. Bakt. u. die Infektionskrankh., Berl. and Wien (1906), 214.

been stated, has been passed successively for over 247 passages from guinea pig to guinea pig without growth on artificial media. (See table of Series 58, p. 296.) These passages have occupied more than a year and a half. The method of infection was carried on as follows:

The first guinea pig of the series was inoculated subcutaneously with one oese of the strain "Pest Virulent" isolated from a human case of plague. Upon the death of the animal two days later, its spleen was removed and a portion of it rubbed over a shaved area on the abdomen of a healthy guinea pig (the next animal of the series). The skin was sometimes slightly scarified with a knife. This method was employed on the third animal, when the second succumbed, and so on without a break throughout the series. In a few instances the autopsy upon the animal and the removal of the spleen did not take place until nearly forty-eight hours after the death of the animal. In a few others, the spleen was removed shortly after the death of the guinea pig and placed on ice and employed for the next inoculation twenty-four hours later. These instances are all noted in the table. They caused no change in the results of the inoculations.

The accompanying table (58) shows that the virulence of the organism during this whole time has not varied. Moreover, its virulence not only for guinea pigs, but for rats and monkeys as well, has remained the same at the end of the series as it was at the beginning; this may be seen from the numerous control animals inoculated from time to time with this strain throughout the series of animal experiments detailed on pages 187 to 288. A glance at Table 58 shows that moderate differences in susceptibility exist in the different guinea pigs, the most susceptible dying in from two to four days after inoculation and the more resistant usually in from seven to eight days. The animals were not always of the same age and weight and for this reason some slight variation in susceptibility also probably occurred.

Only one animal survived for twelve days and but one other for eleven days after the infection. These animals represent the thirty-sixth and one hundred and twentieth passages respectively. The fact that in the thirty-fifth and thirty-seventh passages, the animals died six and four days respectively after infection, and in the one hundred and nineteenth and one hundred and twenty-first passages, four and three days respectively after inoculation, demonstrates that not even a temporary change in virulence in the organisms had occurred at these periods. The length of time the animals lived during the thirty-sixth and one hundred and twentieth passages therefore was probably due to their natural and slightly increased relative resistance to pest infection. Animal number 198 died twenty-four hours after its inoculation. The animal was doubtless sick at the time of the infection.

SERIES 58.—*Showing passage of the strain "Pest Virulent" from guinea pig to guinea pig through 247 animals by cutaneous inoculation from the spleen of one animal to the abdomen of the next without growth on artificial media.*

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
1	1220	Oct. 8	1 oese "Pest Virulent" subcutaneously.	Oct. 10	2	*Bacillus pestis.*
2	1221	Oct. 10	Spleen 1220	Oct. 14	4	Do.
3	1243	Oct. 14	Spleen 1221	Oct. 19	5	Do.
4	1276	Oct. 19	Spleen 1243	Oct. 24	5	Do.
5	1297	Oct. 24	Spleen 1276	Oct. 30	6	Do.
6	1323	Oct. 30	Spleen 1297	Nov. 2	2	Do.
7	1324	do	do	do	2	Do.
8	1354	Nov. 2	Spleen 1324	Nov. 7	5	Do.
9	1355	do	do	Nov. 10	8	Do.
10	1356	do	do	Nov. 7	5	Do.
11	1383	Nov. 7	Spleen 1356	Nov. 13	6	Do.
12	1384	do	do	Nov. 14	7	Do.
13	1435	Nov. 13	Spleen 1383	Nov. 16	3	Do.
14	1436	do	do	do	3	Do.
15	1437	Nov. 14	Spleen 1384	Nev. 17	3	Do
16	1451	Nov. 16	Spleen 1436	Nov. 20	4	Do.
17	1452	do	do	Nov. 19	3	Do.
18	1471	Nov. 17	Spleen 1437	Nov. 20	3	Do.
19	1472	Nov. 19	Spleen 1452	Nov. 24	5	Do.
20	1479	Nov. 20	Spleen 1451	do	4	Do.
21	1480	do	Spleen 1471	Nov. 27	7	Do.
22	1495	Nov. 24	Spleen 1479	do	3	Do.
23	1496	do	Spleen 1472	Nov. 30	6	Do.
24	1497	Nov. 27	Spleen 1495	Dec. 3	6	Do.
25	1498	do	Spleen 1480	Dec. 4	7	Do.
26	1514	Nov. 30	Spleen 1496	do	4	Do.
27	1515	Dec. 3	Spleen 1497	Dec. 7	4	Do.
28	1516	Dec. 4	Spleen 1498	Dec. 13	9	Do.
29	1517	do	Spleen 1514	Dec. 10	6	Do.
30	1557	Dec. 7	Spleen 1515	do	3	Do.
31	1579	Dec. 10	Spleen 1557	Dec. 16	6	Do.
32	1580	do	Spleen 1517	Dec. 15	5	Do.
33	1588	Dec. 13	Spleen 1516	Dec. 20	7	Do.
34	1591	Dec. 15	Spleen 1580	Dec. 19	4	Do.
35	1592	Dec. 16	Spleen 1579	Dec. 22	6	Do.
36	1606	Dec. 19	Spleen 1591	Dec. 31	11	Do.
37	1607	Dec. 20	Spleen 1588	Dec. 24	4	Do.
38	1610	Dec. 22	Spleen 1592	Dec. 27	5	Do.
39	1612	Dec. 24	Spleen 1607	Dec. 28	4	Do.
40	1625	Dec. 27	Spleen 1610	Dec. 31	4	Do.
41	1627	Dec. 28	Spleen 1612	Jan. 3	6	Do.
42	1630	Dec. 31	Spleen 1606	Jan. 4	4	Do.
43	1631	do	Spleen 1625	do	4	Do.
44	1632	Jan. 3	Spleen 1627	Jan. 6	3	Do.
45	1634	Jan. 4	Spleen 1630	Jan. 7	3	Do.
46	1635	do	Spleen 1631	do	3	Do.
47	1643	Jan. 6	Spleen 1632	Jan. 10	4	Do.
48	1645	Jan. 7	Spleen 1634	Jan. 9	2	Do.
49	1646	do	Spleen 1635	do	2	Do.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
50	1648	Jan. 9	Spleen 1645	Jan. 12	3	*Bacillus pestis.*
51	1649	do	Spleen 1646	Jan. 14	5	Do.
52	1650	Jan. 10	Spleen 1643	do	4	Do.
53	1653	Jan. 12	Spleen 1648	Jan. 15	3	Do.
54	1667	Jan. 14	Spleen 1649	Jan. 18	4	Do.
55	1668	do	Spleen 1650	Jan. 19	5	Do.
56	1669	Jan. 15	Spleen 1653	Jan. 17	2½	Do.
57	1673	Jan. 17	Spleen 1669	Jan. 21	4	Do.
58	1674	Jan. 18	Spleen 1667	Jan. 22	4	Do.
59	1676	Jan. 19	Spleen 1668	Jan. 23	4	Do.
60	1697	Jan. 22	Spleen 1673*	Jan. 24	2	Do.
61	1698	do	Spleen 1674	do	2	Do.
62	1700	Jan. 23	Spleen 1676	Jan. 25	2	Do.
63	1702	Jan. 24	Spleen 1697	Jan. 26	2	Do.
64	1703	do	Spleen 1698	Jan. 27	3	Do.
65	1704	Jan. 25	Spleen 1700	Jan. 28	3	Do.
66	1708	Jan. 26	Spleen 1702	Jan. 29	3	Do.
67	1709	Jan. 27	Spleen 1703	Feb. 6	10	Do.
68	1711	Jan. 28	Spleen 1704	Feb. 4	7	Do.
69	1712	Jan. 29	Spleen 1708	Jan. 31	3	Do.
70	1714	Jan. 31	Spleen 1712	Feb. 4	4	Do.
71	1724	Feb. 4	Spleen 1711	Feb. 8	4	Do.
72	1725	do	Spleen 1714	Feb. 7	3	Do.
73	1728	Feb. 7	Spleen 1709	Feb. 10	3	Do.
74	1729	do	Spleen 1725	Feb. 12	5	Do.
75	1745	Feb. 8	Spleen 1724	do	4	Do.
76	1752	Feb. 11	Spleen 1728*	Feb. 13	2½	Do.
77	1755	Feb. 12	Spleen 1729	Feb. 15	3	Do.
78	1756	do	Spleen 1745	Feb. 19	7	Do.
79	1759	Feb. 13	Spleen 1752	Feb. 16	3	Do.
80	1761	Feb. 15	Spleen 1755	Feb. 17	2	Do.
81	1762	Feb. 16	Spleen 1759	Feb. 19	4	Do.
82	1764	Feb. 17	Spleen 1761	Feb. 21	4	Do.
83	1765	Feb. 19	Spleen 1756	Feb. 23	4	Do.
84	1766	do	Spleen 1762	Feb. 24	5	Do.
85	1768	Feb. 21	Spleen 1764	Feb. 26	5	Do.
86	1769	Feb. 23	Spleen 1765	Mar. 2	7	Do.
87	1770	Feb. 24	Spleen 1766	Feb. 27	3	Do.
88	1771	do	do	Mar. 1	4	Do.
89	1772	do	do	Mar. 5	9	Do.
90	1798	Feb. 26	Spleen 1768	Mar. 1	4	Do.
91	1799	do	do	do	4	Do.
92	1800	do	do	Mar. 2	5	Do.
93	1818	Feb. 27	Spleen 1770	Mar. 3	4	Do.
94	1819	Mar. 2	Spleen 1800	Mar. 5	3	Do.
95	1820	Mar. 3	Spleen 1818	Mar. 7	4	Do.
96	1821	Mar. 5	Spleen 1819	Mar. 10	5	Do.
97	1831	Mar. 8	Spleen 1820 †	Mar. 12	4	Do.
98	1833	Mar. 10	Spleen 1821	Mar. 13	3	Do.
99	1834	Mar. 12	Spleen 1831	Mar. 16	4	Do.

*Spleen laid on ice one day before inoculation.

† Spleen placed on ice twenty-four hours before inoculation.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
100	1835	Mar. 13	Spleen 1833	Mar. 16	3	*Bacillus pestis.*
101	1905	Mar. 16	Spleen 1834	Mar. 19	3	Do.
102	1906	----do---	Spleen 1835	----do---	3	Do.
103	1907	Mar. 19	Spleen 1905	Mar. 23	4	Do.
104	1908	----do---	Spleen 1906	Mar. 21	2	Do.
105	1909	Mar. 21	Spleen 1907	Mar. 26	5	Do.
106	1914	Mar. 23	Spleen 1907	----do---	3	Do.
107	1928	Mar. 26	Spleen 1909	Mar. 31	5	Do.
108	1929	----do---	Spleen	----------	--------	Alive.
109	1934	Mar. 31	Spleen 1928	Apr. 3	3	*Bacillus pestis.*
110	1935	Apr. 3	Spleen 1934	Apr. 7	4	Do.
111	1936	Apr. 7	Spleen 1935	Apr. 11	4	Do.
112	1937	----do---	-----do	Apr. 10	3	Do.
113	1938	Apr. 10	Spleen 1937	Apr. 14	4	Do.
114	1939	Apr. 11	Spleen 1936	Apr. 16	5	Do.
115	1940	Apr. 14	Spleen 1938	Apr. 17	3	Do.
116	1941	Apr. 16	Spleen 1939	Apr. 22	6	Do.
117	1946	Apr. 17	Spleen 1940	Apr. 20	3	Do.
118	1947	Apr. 20	Spleen 1946	Apr. 24	4	Do.
119	1948	Apr. 23	Spleen 1941*	Apr. 27	4	Do.
120	1952	Apr. 24	Spleen 1947	May 6	12	Do.
121	1953	Apr. 27	Spleen 1948	Apr. 30	3	Do.
122	1954	Apr. 30	Spleen 1953	May 4	4	Do.
123	1956	May 4	Spleen 1954	May 7	3	Do.
124	1957	May 7	Spleen 1952 †	May 13	6	Do.
125	1958	----do---	Spleen 1956	May 12	5	Do.
126	1959	May 12	Spleen 1958	May 18	6	Do.
127	1962	May 13	Spleen 1957	May 16	3	Do.
128	1997	May 16	Spleen 1962	May 21	5	Do.
129	2005	May 18	Spleen 1959	May 23	5	Do.
130	2052	May 4	Spleen 1997	May 28	7	Do.
131	2055	May 23	Spleen 2005	May 29	6	Do.
132	2060	May 28	Spleen 2052	May 31	3	Do.
133	2061	May 29	Spleen 2055	June 1	3	Do.
134	2062	May 31	Spleen 2060	June 4	4	Do.
135	2065	June 1	Spleen 2060	June 5	4	Do.
136	2066	June 4	Spleen 2062	June 9	5	Do.
137	2067	June 5	Spleen 2065	----do---	4	Do.
138	2073	June 9	Spleen 2066	June 14	5	Do.
139	2074	----do---	Spleen 2067	June 13	4	Do.
140	2123	June 13	Spleen 2074	June 19	6	Do.
141	2124	June 14	Spleen 2073	June 18	4	Do.
142	2140	June 18	Spleen 2124	June 25	7	Do.
143	2141	----do---	-----do	June 27	9	Do.
144	2142	June 19	Spleen 2123	June 25	6	Do.
145	2143	June 25	Spleen 2140	June 30	5	Do.
146	2144	----do---	Spleen 2142	June 29	4	Do.
147	2145	June 29	Spleen 2144	July 2	3	Do.
148	2167	July 3	Spleen 2143	July 6	3	Do.
149	2168	July 2	Spleen 2145	July 7	5	Do.

* Animal 1941 died on Apr. 22. Not autopsied until Apr. 23.
† Animal 1952 died May 6. Not autopsied until May 7.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
150	2174	July 6	Spleen 2167	July 12	6	*Bacillus pestis*
151	2175	July 7	Spleen 2168	July 11	4	Do.
152	2176	July 11	Spleen 2175	July 17	6	Do.
153	2177	July 12	Spleen 2174	July 16	4	Do.
154	2178	July 16	Spleen 2177	July 23	7	Do.
155	2179	July 17	Spleen 2176	July 20	3	Do.
156	2183	July 20	Spleen 2179	July 24	4	Do.
157	2193	July 23	Spleen 2178	Aug. 3	10	Do.
158	2204	July 24	Spleen 2183	July 31	7	Do.
159	2230	July 31	Spleen 2204	Aug. 6	7	Do.
160	2248	Aug. 3	Spleen 2193	do	3	Do.
161	2271	Aug. 6	Spleen 2248	Aug. 11	5	Do.
162	2272	do	Spleen 2230	Aug. 13	7	Do.
163	2283	Aug. 11	Spleen 2271	Aug. 15	4	Do.
164	2293	Aug. 13	Spleen 2272	Aug. 19	6	Do.
165	2303	Aug. 15	Spleen 2283	Aug. 20	5	Do.
166	2315	Aug. 20	Spleen 2293*	Aug. 24	4	Do.
167	2316	do	Spleen 2303	do	4	Do.
168	2322	Aug. 24	Spleen 2315	Aug. 29	5	Do.
169	2323	do	Spleen 2316	Aug. 31	7	Do.
170	2387	Aug. 29	Spleen 2322	Sept. 4	6	Do.
171	2388	Aug. 31	Spleen 2323	Sept. 3	3	Do.
172	2392	Sept. 3	Spleen 2388	Sept. 7	4	Do.
173	2395	Sept. 4	Spleen 2387	Sept. 10	6	Do.
174	2410	Sept. 7	Spleen 2392	Sept. 12	5	Do.
175	2411	Sept. 10	Spleen 2395	Sept. 15	5	Do.
176	2416	Sept. 12	Spleen 2410	Sept. 14	2	Do.
177	2428	Sept. 14	Spleen 2416	Sept. 17	3	Do.
178	2429	Sept. 15	Spleen 2411	Sept. 21	6	Do.
179	2446	Sept. 17	Spleen 2428	do	4	Do.
180	2481	Sept. 21	Spleen 2429	Sept. 24	3	Do.
181	2482	do	Spleen 2446	Sept. 26	5	Do.
182	2483	Sept. 24	Spleen 2481	Oct. 2	8	Do.
183	2485	Sept. 26	Spleen 2482	Sept. 29	3	Do.
184	2496	do	Spleen 2485	Oct. 2	3	Do.
185	2498	Oct. 2	Spleen 2483	Oct. 5	3	Do.
186	2499	do	Spleen 2496	Oct. 4	2	Do.
187	2500	Oct. 4	Spleen 2499	Oct. 5	4	Do.
188	2501	Oct. 5	Spleen 2498	Oct. 8	3	Do.
189	2502	Oct. 8	Spleen 2500	Oct. 13	5	Do.
190	2503	do	Spleen 2501	Oct. 12	4	Do.
191	2507	Oct. 12	Spleen 2503	Oct. 17	5	Do.
192	2560	Oct. 13	Spleen 2502	Oct. 18	5	Do.
193	2583	Oct. 17	Spleen 2507	Oct. 22	5	Do.
194	2585	Oct. 18	Spleen 2560	Oct. 23	5	Do.
195	2586	Oct. 22	Spleen 2583	Oct. 26	4	Do.
196	2587	Oct. 23	Spleen 2585	do	3	Do.
197	2594	Oct. 26	Spleen 2587	Oct. 30	4	Do.
198	2663	Oct. 26	Spleen 2586	Oct. 27	†1	Do.
199	2694	Oct. 27	Spleen 2663	Nov. 5	9	Do.

*Animal 2293 died Aug. 19. Not autopsied until Aug. 20.

† Probably sick, at time of pest inoculation.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
200	2736	Oct. 30	Spleen 2594	Nov. 2	3	*Bacillus pestis.*
201	2750	Nov. 2	Spleen 2736	Nov. 5	3	Do.
202	2764	Nov. 5	Spleen 2694	Nov. 8	3	Do.
201	2765	----do----	Spleen 2750	Nov. 9	4	Do.
202	2827	Nov. 8	Spleen 2764	Nov. 14	6	Do.
203	2828	Nov. 9	Spleen 2765	Nov. 11	2	Do.
204	2841	Nov. 11	Spleen 2828	Nov. 14	3	Do.
205	2858	Nov. 14	Spleen 2841	Nov. 22	8	Do.
206	2859	Nov. 15	Spleen 2827	Nov. 19	4	Do.
207	2865	Nov. 19	Spleen 2859	Nov. 23	4	Do.
208	2866	Nov. 22	Spleen 2858	Nov. 26	4	Do.
209	2869	Nov. 23	Spleen 2865	Nov. 28	5	Do.
210	2895	Nov. 26	Spleen 2866	Nov. 30	4	Do.
211	2921	Nov. 28	Spleen 2869	Dec. 3	5	Do.
212	2923	Dec. 3	Spleen 2921	Dec. 8	5	Do.
213	2949	Dec. 8	Spleen 2923	Dec. 11	3	Do.
214	2954	Dec. 11	Spleen 2949	Dec. 15	4	Do.
215	2961	Dec. 15	Spleen 2954	Dec. 18	3	Do.
216	2965	Dec. 18	Spleen 2961	Dec. 22	4	Do.
217	2968	Dec. 22	Spleen 2965	Dec. 26	4	Do.
218	2970	Dec. 26	Spleen 2968	Dec. 30	4	Do.
219	2978	Dec. 30	Spleen 2970	Jan. 5	6	Do.
220	2982	Jan. 5	Spleen 2978	Jan. 8	3	Do.
221	2987	Jan. 8	Spleen 2982	Jan. 11	3	Do.
222	2990	Jan. 11	Spleen 2987	Jan. 15	4	Do.
223	2993	Jan. 15	Spleen 2990	Jan. 17	2	Do.
224	3009	Jan. 17	Spleen 2993	Jan. 19	2	Do.
225	3010	Jan. 19	Spleen 3009	Jan. 23	4	Do.
226	3016	Jan. 23	Spleen 3010	Jan. 28	5	Do.
227	3026	Jan. 28	Spleen 3016	Feb. 4	7	Do.
228	3039	Feb. 4	Spleen 3026	Feb. 7	3	Do.
229	3048	Feb. 7	Spleen 3039	Feb. 11	4	Do.
230	3058	Feb. 11	Spleen 3048	Feb. 18	7	Do.
231	3079	Feb. 18	Spleen 3058	Feb. 21	3	Do.
232	3086	Feb. 21	Spleen 3079	Feb. 25	4	Do.
233	3098	Feb. 25	Spleen 3086	Mar. 1	4	Do.
234	3099	Mar. 1	Spleen 3098	Mar. 4	3	Do.
235	3100	Mar. 4	Spleen 3099	Mar. 6	2	Do.
236	3101	Mar. 6	Spleen 3100	Mar. 11	5	Do.
237	3104	Mar. 11	Spleen 3101	Mar. 15	4	Do.
238	3107	Mar. 15	Spleen 3104	Mar. 18	3	Do.
239	3112	Mar. 18	Spleen 3107	Mar. 12	3	Do.
240	3115	Mar. 21	Spleen 3112	Mar. 25	4	Do.
241	3116	Mar. 25	Spleen 3115	Mar. 30	5	Do.
242	3118	Mar. 30	Spleen 3116	Apr. 3	4	Do.
243	3120	Apr. 3	Spleen 3118	Apr. 8	5	Do.
244	3121	Apr. 8	Spleen 3120	Apr. 13	5	Do.
245	3134	Apr. 13	Spleen 3121	Apr. 19	6	Do.
246	3137	Apr. 19	Spleen 3134	Apr. 24	5	Do.
247	3138	Apr. 24	Spleen 3137	Apr. 29	5	Do.

As mentioned above, Otto showed that it was not possible to increase the pathogenicity of strains of pest bacilli of moderate virulence by repeated passages through animals. The strains with which he performed his experiments were all capable of killing guinea pigs in moderate doses at the time of the commencement of the work.

I have attempted to increase the virulence of a more attenuated culture of the pest bacillus (one which in large doses was not capable of killing guinea pigs) by experiments performed on monkeys. The strain "Avirulent Manila" was employed for the inoculations. This organism was chosen because it had at an earlier time possessed a much greater virulence and because its pathogenicity had been reduced artificially. (See p. 310.) It was therefore interesting to see if the virulence could be reclaimed.

Experiments of this nature were first undertaken with the idea of ascertaining how long the attenuated organisms would remain alive in the tissues after subcutaneous inoculation. The technique of the experiments was as follows:

> The abdomen of a monkey was first shaved and a suspension of the organism inoculated subcutaneously. The skin was then carefully massaged until the fluid was apparently completely absorbed. After varying periods of time, the skin of the abdomen was scrubbed several times with ether and alcohol and a small incision made with a sterile knife through the dermis. Cultures were then taken from the drops of blood or from the pus which escaped from the incised wound.

Usually, when the injection was made beneath the skin of the abdomen, a few hours later about the point of inoculation an œdematous swelling appeared which did not entirely disappear for forty-eight hours. In an earlier series of experiments made with a slightly more attenuated pest culture, it was found that the organisms were still very numerous in the tissues six to eight hours after the inoculation, after which time they gradually disappeared so that cultures made twenty-four hours subsequent to the injection generally, though not always, remained sterile. It seemed probable that the more resistant organisms were those which remained alive in the tissues for the longest time and that there was a true "survival of the fittest." An attempt was therefore made to ascertain if it would be possible to increase the pathogenicity of a slightly more virulent culture by this same procedure. As soon as the cultures made on agar from the incision in the animal developed, they were inoculated subcutaneously into a second monkey and so on throughout the series. The strain used for this series of experiments ("Avirulent Manila") was so attenuated at the time of their commencement that one 24-hour agar slant culture usually, but not invariably, caused death from subacute plague infection in a guinea pig of about 250 grams weight. This culture was passed through twenty-five monkeys according to the method above described. At the end of this time its virulence

was found to be practically unchanged. The details of all the passages are given in Series 28 which follows. The monkeys employed were all of small size. The cultures were made from the animal, from one and one-half to twenty-four hours after the time of the inoculation. In a few instances in which the quantity of inoculated bacteria was large, pus formation about the point of injection was found to have occurred on incising the skin from twenty-one to twenty-four hours after the time of the infection of the animal. However, the organisms in the pus did not prove infectious when inoculated into guinea pigs in a considerable amount.

Therefore, my attempts to increase the virulence of a very virulent pest strain or to reclaim that of a more attenuated one have been entirely unsuccessful, the organisms in each instance having retained a very stable virulence throughout.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila."*

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1581	Dec. 11 at 9 a. m.	One 24-hour agar slant culture suspended in 1 cc. bouillon.	Dec. 11 at 10.30 a. m. after 1½ hours.	Almost uniform growth of colonies over surface of slant.	Alive	A number of bacilli still present.	
1582	do	do	Dec. 11 at 1.30 p. m. after 4½ hours.	1 tube 114 colonies; in another 24 colonies.	do		
1589	Dec. 13 at 9 a. m.	One 48-hour agar culture obtained from monkey No. 1581 and one 48-hour agar culture obtained from monkey No. 1582, containing 114 colonies. These two cultures together suspended in 1 cc. of bouillon.	Dec. 13 at 2 p. m. after 5 hours.	5 cultures made. All developed innumerable colonies. The colonies appear as numerous as they usually do in colonies from the heart's blood of guinea pigs dead of pest infection.	Dead Feb. 5.	A few bacilli still present.	No bacilli in smears from spleen. 2 cultures from spleen negative. Animal much emaciated. No local lesion about point of inoculation apparent. Wound perfectly healed. Cause of death obscure.
1590	Dec. 15 at 10.20 a. m.	5 of the agar cultures (48 hour) which developed from monkey No. 1589 all together suspended in 1 cc. bouillon. The 5 cultures = about 1⅓ full-grown agar slant cultures.	Dec. 15 at 4.30 p. m. after 6 hours.	Cultures show very numerous colonies of pest bacilli. Almost the whole surface of the slant covered.	Dead Jan. 17.*	Typical pest bacilli present.	Miliary and conglomerate tubercles in liver, spleen and lungs. Microscopical specimens show no plague bacilli. A few tubercle bacilli in smears from lungs. 2 cultures from the spleen and 2 from the heart, negative.*

*This is the only monkey found to be suffering with tuberculosis during the course of this research. Tuberculosis is very rare in monkeys in the Philippine Islands.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1593	Dec. 18 at 9 a. m.	One 3-day agar slant culture from monkey No. 1590 suspended in 1 cc. bouillon.	Dec. 18 at 4.30 p. m. after 7½ hours.	Colonies nearly cover whole surface of slant.	Alive and well.		Although the injection was made rather deeply into the abdominal muscles and all the fluid injected was quickly absorbed, nevertheless at 4.30 p. m., when the cultures were taken, an area of considerable œdema had developed over the abdomen about the region of the inoculation.
1608	Dec. 20 at 10.30 a. m.	1 agar slant 48-hour culture from monkey No. 1593.	Dec. 20 at 4.30 a. m. after 6 hours.	Very numerous colonies	Dead Jan. 9.		In smears from the spleen it appeared that a few swollen bacilli were present. However, in two cultures from this organ in which a large amount of material was inoculated no growth took place.
1609	Dec. 20 at 10.30 a. m.	One 48-hour agar slant culture from monkey No. 1593 in 1 cc. bouillon.	Dec. 21 at 10.30 a. m. after 24 hours.	Culture negative	Dead Jan. 15		No evidences of pest infection. 2 cultures from spleen and 2 from heart negative.
1611	Dec. 22 at 10 a. m.	Two 48-hour cultures from monkey No. 1609 in 1 cc. bouillon.	Dec. 22 at 4 p. m. after 4 hours.	Numerous colonies developed.	do		Much emaciated. Nothing to suggest plague at autopsy. 2 cultures from spleen negative.
1626	Dec. 27 at 11 a. m.	One 5-day culture from monkey No. 1611 in 1 cc. bouillon.	Dec. 27 at 4.15 p. m. after 5 hours.	About 10 or 12 colonies on entire culture.	Alive		

1629	Dec. 29 at 11 a. m.	One 48-hour culture from monkey No. 1626, equal to about $\frac{1}{10}$ of a full agar slant in 1 cc. bouillon.	Dec. 29 at 3 p. m. after 4 hours.	Fair number of colonies developed.	do	Bacilli still present.	
1633	Jan. 3 at 10.30 a. m.	One 5-day culture from monkey No. 1629 in 1 cc. bouillon.	Jan. 3 at 3.30 p. m. after 5 hours.	Culture from drop of blood developed only a few colonies. Culture from deeper tissues developed very numerous colonies. Nearly whole surface of agar slant covered.	do		
1642	Jan. 5 at 11.30 a. m.	One 48-hour culture full grown slant from No. 1633 in 0.5 cc. bouillon.	Jan. 5 at 4.30 p. m. after 5 hours.	Culture from incision extending into muscle. Very rich growth of pest bacilli.	do		
1644	Jan. 6 at 10 a. m.	One 16-hour culture from monkey No. 1642 in 1 cc. bouillon.	Jan. 6 at 1 p. m. after 3 hours.	Numerous colonies	do		
1647	Jan. 8 at 10.30 a. m.	One 48-hour agar slant from monkey No. 1644 in 1 cc. bouillon.	Jan. 8 at 4 p. m. after $5\frac{1}{2}$ hours.	do	do		Jan. 10, a guinea pig (No. 1652) was inoculated subcutaneously with $\frac{3}{4}$ agar slant (second generation from monkey No. 1647) suspended in 1 cc. bouillon. Animal remained well. Evidently no marked increase in virulence of the organisms.
1651	Jan. 10 at 10.30 a. m.	One 48-hour agar slant from monkey No. 1647 in 1 cc. bouillon.	Jan. 10 at 4 p. m. after $5\frac{1}{2}$ hours.	Rich growth of pest bacilli. Very nearly whole surface of slant covered.	do		

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1654	Jan. 12 at 10 a. m.	One and one-half 48-hour agar slant from monkey No. 1651 in 1 cc. bouillon.	Jan. 12 at 4 p. m. after 6 hours.	Rich growth	Alive		
1670	Jan. 15 at 10.30 a. m.	One 3-day culture from monkey No. 1654 suspended in 1 cc. bouillon.	Jan. 15 at 4.30 p. m. after 6 hours.	do	Alive and well.	Cover slip	
1672	Jan. 16 at 4 p. m.	One and one-half 24-hour culture from monkey No. 1670 suspended in 1 cc. bouillon.	Jan. 17 at 9 a. m. after 17 hours.	do	do	Numerous organisms still present. Many enclosed in leucocytes.	
1675	Jan. 18 at 4 p. m.	One 30-hour agar slant culture from monkey No. 1672 suspended in 1 cc. bouillon.	Jan. 19 at 9.30 a. m. after 17½ hours	Cultures from drops of blood which oozed from the wound developed but very few colonies. Cultures from deeper tissues showed numerous colonies but these colonies became contaminated and could not be used for further inoculation.	Alive	Microscopical preparation from the blood which oozed from the incision showed no bacilli. On making a deeper incision and securing other specimens many bacteria were found. A large number were engulfed in leucocytes.	
1699	Jan. 22 at 11.45 a. m.	One 48-hour agar slant culture from monkey No. 1675, second generation, in 1 cc. bouillon.	Jan. 22 at 4 p. m. after 4 hours.	Rich growth	Alive and well.		

1701	Jan. 23 at 4 p. m.	One 24-hour agar culture from monkey No. 1699 in 1 cc. bouillon.	Jan. 24 at 10 a. m. after 18 hours.	do	do	Microscopical smears show numerous pest bacilli. Many engulfed in leucocytes.	
1706	Jan. 25 at 3 p. m.	One 24-hour agar culture from monkey No. 1701 in 1 cc. bouillon.	Jan. 26 at 9 a. m. after 18 hours.	Rich growth. Colonies almost entirely confluent.	do	Few organisms present.	
1710	Jan. 27 at 1 p. m.	One 24-hour agar culture from monkey No. 1706 in 1 cc. bouillon.	Jan. 28 at 10 a. m. after 21 hours.	Rich growth	do		
1713	Jan. 29 at 4 p. m.	One 24-hour agar culture from monkey No. 1710 in 1 cc. bouillon.	Jan. 30 at 9. a. m. after 17 hours.	do	do	A few bacilli seen. All extracellular.	
1715	Jan. 31 at 4.30 p. m.	One 24-hour agar culture from monkey No. 1713 in 1 cc. bouillon.	Feb. 1 at 9 a. m. after 16½ hours.	do	do	Very numerous pest bacilli in smear from blood from incision.	
1723	Feb. 2 at 3 p. m.	One 24-hour agar culture from monkey No. 1715 in 1 cc. bouillon.	Feb. 3 at 9 a. m. after 18 hours.	do	do	Numerous pest bacilli in smears from incision. Many within leucocytes.	
1726	Feb. 4 at 9.30 a. m.	One and one-half 24-hour cultures from monkey No. 1723 in 1 cc. bouillon.	Feb. 5 at 9 a. m. after 23 hours.	do	do	Microscopical preparation showed many pus cells containing numerous pest bacilli. Also free organisms.	On Feb. 5, on incision of the skin over the point of inoculation, a small pocket of grayish (not yellow) pus was encountered. The abdomen of guinea pig No. 1727 was shaved and scarified with a scalpel and several loops of this pus and blood rubbed over the scarified area. The animal remained well.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1730	Feb. 7 at 10.30 a. m.	Two 48-hour agar slant cultures from monkey No. 1726 in 1 cc. bouillon.	Feb. 8 at 8.45 a. m. after 10 hours.	Rich growth	Alive and well.	Very numerous pest bacilli in smear of blood from incision; both intra-and extra-cellular; numerous pus cells.	
1746	Feb. 10 at 12.30 a. m.	One 48-hour full agar slant culture from monkey No. 1730 in 1 cc. bouillon.	Feb. 11 at 9.30 a. m. after 21 hours.	do	do	Numerous pest bacilli in pus which oozed from incision. Many within leucocytes.	On Feb. 11, on incision over the point of inoculation where a distinct swelling had occurred, considerable pus escaped.
1757	Feb. 12 at 9.30 a. m.	One and one-half 24-hour agar slant cultures from monkey No. 1746 in 1 cc. bouillon.	Feb. 13 at 9.30 a. m. after 24 hours.	do	do	Numerous pest bacilli in drops of blood from incision.	A considerable swelling developed about the point of inoculation and on Feb. 13, on incising this area to make cultures, blood, pus, and serum escaped. About 0.2 cc. of this material was drawn up in a syringe and inoculated beneath the skin of the abdomen of guinea pig No. 1758. The animal remained entirely well.
*1760	Feb. 14	One 30-hour full agar slant culture from monkey No. 1757 suspended in 1 cc. bouillon, inoculated subcutaneously.			do		Evidently there has been no increase of virulence.

*Guinea pig, 240 grams' weight.

ARTIFICIAL ATTENUATION OF THE PLAGUE BACILLUS.

In spite of the facts which have been brought out in the previous discussion, nevertheless it is true that the virulence of different pest strains as they occur in nature varies greatly, the numerous cultures isolated from different epidemics and different cases of human plague frequently exhibiting marked variation in pathogenicity in animal experiments. It is also true that there are a number of references in the literature which point to the fact that certain strains of pest bacilli, after growing for a few generations on artificial culture media, unaccountably lose their virulence. It therefore is not my purpose to argue that under certain conditions the virulence of the pest bacillus may not vary from time to time and that it may not frequently, under some conditions in nature, become attenuated and under others, such as its passage from animal to animal, even be increased. However, any such change as the latter is very unusual and it does not occur under ordinary circumstances. The important point which I wish to emphasize in the behavior of this organism in its passage from animal to animal is its stability of virulence, and this quality is also possessed to a remarkable degree by the organism on artificial culture media.

Virulent strains of the pest bacillus when grown upon artificial media usually suffer no change in their pathogenicity even after long periods of time and after numerous generations. Cultures sealed in test tubes and protected from light and heat have been found to retain their full virulence for several years. Thus Maassen found no change of virulence in cultures after two years and N. K. Schultze [110] observed that cultures in bouillon were fully virulent even after four years. Attempts to attenuate the virulent organism in cultures have also demonstrated its stability in pathogenicity and have usually resulted unsuccessfully.

Albrecht and Gohn [111] reported that the pest bacillus could resist a continued temperature of 36° C. for only about fourteen days without loss of virulence, and that after this period a rapid diminution occurred. However, these results have not been confirmed by any other observers. The German Plague Commission [112] suspended agar cultures of the organism in bouillon and exposed them to a temperature of 51° C. for varying periods of time. They found that while many of the organisms were killed by this treatment, those which remained alive had retained their full virulence. They also attempted to secure attenuation of the organism by exposing it to carbolic acid, but these experiments were also unsuccessful.

Kolle and Otto [113] found that by growing the organism at a continuous temperature of 40° to 41° C. a further attenuation was sometimes possible in

[110] *Centrbl. f. Bakteriol.* (1901), **29**, Abt. I, 169.
[111] *Loc. cit.*
[112] *Loc. cit.*
[113] *Deutsche med. Wchnschr.* (1903), **29**, 493.

cultures which already were of a low grade of virulence.[114] Hetsch has experimented extensively with the question of the artificial attenuation of the plague organism. He found that prolonged growth of the bacilli at temperatures ranging from 40° to 45° C. caused no attenuation of the surviving organisms. Cultivation of the germ in an insusceptible animal such as the frog (in its dorsal lymph sac) also caused no lowering of the virulence. After experimenting with a number of chemical substances, particularly with various staining reagents, he found that the most satisfactory means of attenuation consisted of cultivating the organism in flasks of bouillon to which increasing amounts of alcohol were gradually added.

I have been successful in attenuating two strains of pest bacilli by employing the method recommended by Hetsch. My cultures in the alcoholic bouillon, in addition, were frequently grown at a temperature of from 41° to 43° C. The strains used for the experiments were those designated as "Pest Virulent" and "Pest Avirulent Manila." The organism was first inoculated into a 50 cubic centimeter flask containing bouillon; after forty-eight hours' growth at 30° C. the alcohol was added and the flask placed at a temperature of 41° to 43° C. for a period usually of three weeks. Cultures from the flask were then inoculated upon agar for many generations, a fresh one being made each day, sometimes for one or two weeks, and the organism usually cultivated at the same high temperature. Beginning with 0.1 cubic centimeter of absolute alcohol and 50 cubic centimeters of bouillon, the amount of the former was gradually increased in successive cultures up to 5 cubic centimeters in 50 cubic centimeters of bouillon. In some instances the alcohol in the flask was increased each week and in others every few days. The organisms in the flask containing the larger amounts of alcohol frequently perished entirely and in other instances, when inoculations were made from them upon agar only, a few isolated colonies developed in the medium. If the former took place, the cultures were obviously lost and the last step of the attenuation had then to be repeated with the previous transplant of the more virulent organism.

This method of attenuation does not give quick results, and as Hetsch admits, it is frequently not satisfactory for diminishing the virulence of the plague organism. Moreover, it sometimes appears to be very inefficient in bringing about this change when the strains are of very great virulence. However, it would appear to be the most successful method which has yet been discovered. The time during which the experiments were carried on in the attenuation of the strain "Pest Avirulent Manila" was a little over seven months, and in the strain "Pest Virulent" about seventeen months.

[114] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 443.

At the time of the beginning of the attenuation the strain "Pest Avirulent Manila," when inoculated subcutaneously, killed guinea pigs of from 200 to 300 grams weight in doses of about 2 oesen. At the time the experiments were discontinued, the lethal dose for guinea pigs of the same weight was about one agar slant culture, although the animal sometimes even survived such a dose.

The organism during the time of its attenuation was grown for five different periods in the bouillon containing the different percentages of alcohol. Series 59 (p. 312) gives the details of the change in virulence.

At the time of the beginning of the experiments in the attenuation of the strain "Pest Virulent" the organism possessed a virulence so great that a portion of a spleen of a guinea pig which had died from infection with it, when it was rubbed over the abdomen of a second guinea pig, always caused the death of the animal from acute plague infection, and 5 oesen of a suspension (prepared by adding 5 cubic centimeters of saline solution to a 24-hour agar slant culture of the organism) when rubbed over a shaved and scarified area on the abdomen of a guinea pig, also invariably caused it to succumb to plague. After the virulent strain had been grown for nearly three months in the alcoholic bouillon, ½ oese of a 24-hour agar culture of the organism when injected subcutaneously into a guinea pig, still caused its death; after the experiment had been carried on for eleven months the virulence of the organism was found to be such that 2 oesen of the 48-hour agar culture, when rubbed over a shaved and scarified area on the abdomen of a guinea pig, or the same amount injected subcutaneously, no longer caused the death of the inoculated animals. However, ½ agar slant after two months more of attenuation, invariably caused fatal infection. At the time of the termination of the experiments which lasted for about seventeen months, the virulence was so reduced that ½ agar slant culture of the organism, when injected subcutaneously into guinea pigs, did not cause the death of more than 40 per cent of the animals so inoculated, the remaining 60 per cent remaining alive and well. At the termination of the experiment the same original strain (unattenuated) which had been kept in a sealed tube and protected from light during the same period of time, was grown for two successive transplants upon fresh agar and its virulence again tested. It was found that 0.1 oese of this culture, when rubbed over the scarified abdomen of a guinea pig, still caused the death of the animal, showing that no practical decrease in the virulence of the organism had as yet occurred. The details of the attenuation of the strain are recorded in the series of experiments numbered 60.

SERIES 59.—*Showing the attenuation of the strain "Pest Avirulent Manila," in guinea pigs.*

Animal No.	Inoculated.	Result.	Autopsy.
1164	June 28, two oesen strain "Pest Avirulent Manila" subcutaneously.	Dead July 3, after 5 days.	Numerous pest bacilli in smears from spleen.
1166	July 10, two oesen strain "Pest Avirulent Manila" subcutaneously.	Dead July 16, after 6 days.	Do.
1450	Nov. 14, one-half agar slant culture "Pest Avirulent Manila" (attenuated 5 months) subcutaneously.	Alive and well.	
1578	Dec. 9, one 48-hour agar slant culture "Pest Avirulent Manila" (attenuated 6 months) subcutaneously.	Dead Dec. 18, after 9 days	Few involution forms of plague bacilli in the heart's blood. Six colonies developed in one culture and none in the second.
1655	Jan. 12, three-fourths 24-hour agar slant culture "Pest Avirulent Manila" (attenuated 7 months) subcutaneously.	Alive and well.	
1671	Jan. 15, one 48-hour agar slant culture "Pest Avirulent Manila" (attenuated 7 months) subcutaneously.	Dead Jan. 29, after 14 days.	Subacute pest.
1751	Feb. 11, one 30-hour agar slant "Pest Avirulent Manila" (attenuated 7½ months) subcutaneously.	Alive and well.	

SERIES 60.—*Showing the attenuation of the strain "Pest Virulent," in guinea pigs.*

Animal No.	Inoculated.	Result.	Autopsy and remarks.
1497	Nov. 27, abdomen shaved and rubbed with spleen of guinea pig No. 1495, just dead of plague from inoculation of the strain "Pest Virulent."	Dead Dec. 2, after 5 days.	Numerous pest bacilli in smears from spleen.
1763	Feb. 16, one-half oese "Pest Virulent" (attenuated 3 months) subcutaneously.	Dead Feb. 20, after 4 days.	Pest.
2068	June 7, one oese "Pest Virulent" (attenuated 6 months) subcutaneously.	Dead June 12, after 5 days.	Do.
2069	----do----	Dead June 14, after 7 days.	Do.
2070	----do----	Dead June 12, after 5 days.	Do.
2071	----do----	Dead June 11, after 4 days.	Do.
2072	----do----	Dead June 14, after 7 days.	Do.
2194	July 23, one oese "Pest Virulent" (attenuated 8 months) subcutaneously.	Alive and well.	
2195	----do----	----do----	
2196	----do----	Dead Aug. 4, after 12 days.	Subacute pest.
2592	Oct. 25, two oesen "Pest Virulent" (attenuated 11 months) subcutaneously.	Alive and well.	
2593	----do----	----do----	

SERIES 60.—*Showing the attenuation of the strain "Pest Virulent," etc.*—Continued.

Animal No.	Inoculated.	Result.	Autopsy and remarks.
3029	Jan. 29, one agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Dead Feb. 5, after 7 days.	Large suppurating bubo in abdominal wall.
3030	do	Dead Feb. 4, after 6 days.	Large cyst-like bubo.
3031	Jan. 29, one-half agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Dead Feb. 11, after 13 days.	Advanced pest pneumonia.
3032	do	Dead Feb. 9, after 11 days.	Subacute pest.
3033	Jan. 29, one-fourth agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Alive and well	
3034	do	do	
3139	Apr. 25, one-half agar slant culture "Pest Virulent" (attenuated 17 months) subcutaneously.	Dead May 6, after 11 days.	Large bubo. Subacute pest.
3140	do	Alive and well	
3141	do	do	
3142	do	do	
3143	do	do	
3144	do	do	
3145	do	Dead May 6, after 11 days.	Subacute pest.
3146	do	Dead May 3, after 8 days.	Do.
3147	do	Dead Apr. 26, after 1 day.	Not of pest infection.
3148	do	Alive and well	
3149	Apr. 25, one-tenth oese "Pest Virulent" (original) rubbed over abdomen.	Dead Apr. 30, after 5 days.	Acute pest infection.

STABILITY OF VIRULENCE OF THE PLAGUE BACILLUS.

These experiments, relating to the artificial attenuation of the pest bacillus in cultures, as well as those performed on animals with the view of increasing the pathogenicity of this organism all demonstrate its great stability of virulence.

In respect to this phenomenon, it is interesting to compare the behavior of the cholera organism with that of the pest bacillus, both in its growth on artificial media and in successive passages through the animal body. The cholera organism if left for a few months on culture media such as agar, soon loses its virulence and indeed, frequently dies out, largely owing to the autolytic digestion of the bacteria which takes place. If, instead of allowing the spirillum to remain in the original tube in which it was inoculated, it is transplanted for a number of times on agar, a rapid loss of virulence is also obtained. On the other hand, this virulence can quickly again be increased by successive passages of the culture through a series of a species of animal such as the guinea pig, and in

from fifteen to twenty-five successive passages the maximum virulence of the spirillum can usually be reached if the attenuation has not been too great. Again, if such a culture which has attained its maximum virulence be left upon artificial media for a few weeks, the virulence becomes greatly reduced. This process of increasing and of decreasing the virulence of the cholera organism may be repeated at will.

On the other hand, we have seen with the pest bacillus that it is impossible to reproduce these phenomena. The pest bacillus does not easily undergo autolysis in old agar cultures and it produces no ferments which are capable of causing its own destruction. Virulent pest cultures, whether they remain continuously in the original agar culture upon which they were first inoculated or are transplanted from culture to culture, remain alive and frequently retain their full virulence, although many instances of spontaneous loss of this property occur. Strains of the organism of moderate virulence, but which still kill guinea pigs, can not be rendered perceptibly more virulent by repeated passages from animal to animal, unless perhaps it be under certain special conditions and the same applies to avirulent strains of the organism which rarely kill the animal except in very large doses.

This evidence is particularly interesting from an epidemiological standpoint, when we compare epidemics of cholera with those of plague. With the former disease, the epidemic—no matter which type it may later assume—probably begins either by the introduction of the virulent organism into a new district by a sick individual who has traveled from a region where the disease is already epidemic, or by a few cases of infection in the neighborhood of the new district, which are usually of a mild character and may entirely fail to attract attention. In case the water supply of the region becomes infected from some of these cases, the organism having increased in virulence in its passage through the more susceptible individuals with which it has come in contact, the epidemic may assume an explosive character which is very marked and thousands of people may be stricken within a day or two. If the epidemic does not extend through the water supply, but by direct or indirect contact with cases of infection, a better opportunity for its study is usually afforded. It may then frequently be observed, although obviously this is not always the case, that as the epidemic extends the virulence of the cases increases to a maximum and then as the number of cases slowly subsides, the severity also usually becomes reduced, so that within a short period of time, at most within a year or two, the epidemic has either reached its maximum severity or has entirely disappeared. Such outbreaks of cholera would appear partially to be due (leaving meteorological influences, relation to soil, dissemination of the organism, etc., out of the question), to a rise of virulence of the cholera spirillum which is brought about by the fact that the more or less attenuated

organism has come in contact with a number of particularly susceptible individuals and the period of the epidemic, under certain conditions at least, seems to be limited particularly by the virulence of the organism and by the number of susceptible individuals which continue to be infected with it. As the virulence of the organism diminishes, the number of cases of cholera in a community will diminish. Avirulent cholera organisms, when grown on culture media in the laboratory, are practically harmless when ingested by all but very susceptible human beings and the same is probably true of the more attenuated spirilla which occur in nature. It would then appear that the instability of the virulence of the cholera organism must frequently play an important rôle in determining the character and duration of the cholera epidemic.

How different is the picture in at least some plague epidemics, such, for example, as that which has been witnessed in India for the past ten years and in which the number of deaths reported each year has almost steadily increased from 1897 to 1904–5. The reported deaths in India from this disease, according to the Indian Medical Gazette of July, 1906, are as follows:

	Deaths.
Up to end of 1897	57,965
1898	118,103
1899	134,102
1900	91,627
1901	273,679
1902	577,427
1903	851,263
1904	1,022,300
1905	950,863
1906 to end of April	170,000

It can not be argued that the disease is becoming any more or any less virulent from year to year, judging from the death rate, for we know that it is still spreading and that it has not even yet extended over the whole of India. Burma has escaped until within the last few months, but is now suffering very severely.

Knowing as we do from laboratory experiments that the plague bacillus is frequently extremely stable in its virulence in nature and that it may not easily become attenuated under artificial conditions, knowing also that the epidemic of this disease in India has not decreased in a period of ten years, the outlook for the extermination of the malady in that country by any other means than by the exhaustion of suitable susceptible individuals and animals does not now seem to be hopeful.

It is for this reason that I agree with Professor Haffkine, Colonel Bannermann and many others in the idea that it is certain that protective inoculation against plague must become one of the most effective means

that can be employed in India for the extermination of the malady. And while in combating this disease the ordinary hygienic measures should in my opinion certainly not be neglected and special attention should be given to disinfection and particularly to the destruction of rats and fleas, yet it must be borne in mind that in spite of all these precautions plague may continue to exist as it has and one might almost say will continue to exist in India until the larger portion of the susceptible population is either protected artificially by vaccination or naturally by an attack of the disease, or until the agents which transmit the malady become exhausted.

As the disease spreads and the organism becomes widely disseminated in new districts and new countries, if the same conditions for the transmission of the malady are to be encountered in the newly infected regions, we must not be surprised if the same virulence and the same high mortality continue. Only through the destruction of the bacillus and of the means by which it is conveyed to man (rats, fleas, etc.) and through the exhaustion of the supply of the more susceptible human beings, does it appear that some epidemics of plague may be limited. As long as the organisms in these epidemics are sufficiently disseminated and properly conveyed to man, the epidemics may continue for indefinite periods. In other words, there is not the same hope of a plague epidemic wearing itself out, so to speak, as there is of a cholera epidemic doing so.

On the other hand, it is well known that the severe epidemics of the disease may be preceded by sporadic cases of mild plague, and that glandular swellings in cases free of fever have also frequently been reported to exist, before a more general outbreak of plague appears in malignant form. The malady may also increase in virulence if it occurs in the same locality in successive years, as was witnessed in the epidemic at Bombay in 1896–97. We also know, as has been pointed out by Simpson,[115] that plague may begin in a new locality, then pass into a very virulent variety and after reaching epidemic proportions, gradually return to a mild form of the disease; or, on the other hand, that some epidemics may be mild and others malignant throughout.

In this connection it is interesting to observe that J. F. Payne[116] goes so far as to distinguish two distinct strains of plague: First, the Eastern Asiatic strain, distinguished by—

(1) The frequent occurrence of epidemics of *Pestis minor* or mild plague; (2) the absence of any observed connection with the epizoötic disease of rodents; (3) more self-limited epidemics; (4) consequently less marked power of extension, for the eastern Asiatic epidemics have not in modern times traveled very far; (5) on the whole less virulence and a lower percentage mortality.

[115] Treatise on Plague (1905), 159. Cambridge.

[116] Allbutt & Rolleston, System of Medicine (1907), 2, 388.

Second, the Indo-China strain which, in contrast to the first, is distinguished by—

(1) An almost invariable connection with great mortality among rats and less frequently among other animals; (2) less frequent occurrence of epidemics of *Pestis minor*, although it is possible that further observations may show that these are commoner than has been supposed; (3) remarkable power of extension as shown in its spread over a large part of India and conveyance to many other countries; (4) in general, more intense virulence and higher case mortality; (5) the pneumonic form accompanied by hæmoptysis is much commoner.

However, the variations in virulence of the disease are dependent somewhat upon the differences in the susceptibility of the individual attacked and are not entirely connected with the degree of virulence of the organism, which as we have seen from a discussion of this subject, may change under some of the physical conditions it meets in nature and in certain of the passages through the animal body.

Therefore, it must unquestionably be admitted that the pest bacillus may under certain conditions become attenuated many times during the course of an epidemic, and under certain others regain its virulence. Nevertheless, the general stability of its virulence must be recognized, and I believe that it is this quality which may markedly influence the nature and course of at least some epidemics of plague.

The plague organism in nature is not very resistant and easily becomes destroyed under certain conditions, but it frequently does not easily become attenuated, either in the animal body or outside of it.

RELATION BETWEEN THE VIRULENCE OF THE PLAGUE BACILLUS AND ITS IMMUNIZING POWER.

The question of the relation between the virulence and the immunizing power of different strains of the plague bacillus obviously is an important one in its bearing upon the subject of protective inoculation, particularly with reference to that form in which the living, attenuated organism is employed. The subject of virulence in relation to the immunizing power of microörganisms in general has attracted considerable attention during the past few years. Pfeiffer, Friedberger [117] and myself [118] found that with cholera spirilla a greater immunity was obtained with the more virulent organism. Pfeiffer and Friedberger employed four strains in their investigations. My experiments were carried on with two strains of cholera spirilla of widely different virulence, and I was able conclusively to show that the virulent organism upon inoculation produced a higher immunity and at the same time bound a greater number of amboceptors in a cholera immune serum

[117] *Berl. Klin. Wchnsch.* (1902), **39**, 581.

[118] *Publications of the Bureau of Government Laboratories*, Manila (1904), No. 21; 1 *J. Exp. Med.* (1905), **7**, 229.

than did the avirulent one. At the time of the publication of these experiments I stated that "these conclusions apply to the two strains of cholera spirilla employed in the foregoing experiments. Whether they will also hold good with other strains of this spirillum or for micro-organisms in general must be decided by further experimental work."

Wassermann,[119] in studying the question with typhoid bacilli found that two strains of the organism which possessed a greater immunizing power and a greater power of binding amboceptors than a third strain, were nevertheless less virulent. From these experiments one would conclude that the immunizing power depended rather upon the binding power of the organism than upon its virulence. During the past year Meinicke, Jaffé and Flemming,[120] have performed careful and much more extensive experiments with cholera spirilla, their investigations relating to the question of virulence with reference to the power to bind amboceptors and to immunize. While in some instances they found that a virulent cholera culture was able to bind more amboceptors than an avirulent one, in the great majority of their experiments they were able to show that the binding power was independent of virulence, since in many instances the less virulent culture bound a greater number of amboceptors than the more virulent one.

Their experiments referring to the question of the relation between virulence and immunizing power are not numerous, as the authors admit. This seems to me to be unfortunate, for by means of their methods of examination and the large number of strains which they studied they were in a position to solve this problem conclusively. In one experiment they were able to show that, in one instance at least, a much attenuated culture was in a rabbit able to give rise to a serum of higher value than resulted from the use of a very virulent culture. Nevertheless, it would appear that an individual variation in immunity in the rabbit immunized with either the virulent or the avirulent organism, might also account for the difference in immunity obtained, and therefore it would appear to be injudicious to draw decided conclusions from this one result. Moreover, it may be seen in the table which Meinicke, Jaffé and Flemming compiled, and the fact is also emphasized in the text of their article, that with the small doses inoculated, 0.1 and 0.01 oese, there was very great variation in the value of the sera produced in the different rabbits. Thus, of two animals, each inoculated with 0.01 oese of the same culture, one furnished a serum showing a bactericidal value of 1:400 and the other of 1:1,000. However, it must be admitted that the experiments of these authors not only show that with the cholera organism binding power may be independent of virulence but they also suggest very strongly, if they do not prove conclusively, the fact that the immunizing power also may be independent of virulence.

[119] Festschrift Robert Koch (1903), 503.

[120] *Ztschr. f. Hyg. u. Infectionskrank.*, Leipz. (1906), **52**, 416.

Pfeiffer[121] found that in immunization against plague, the degree of immunity produced depended not only upon the dose of the killed pest culture but also upon the degree of virulence of the killed organism, and concluded that the immunizing effect of pest bacilli is, up to a certain extent, proportional to the virulence of the culture employed. Kolle and Martini[122] found that the less virulent strains of pest bacilli were agglutinated by an immune serum in higher dilutions than more virulent ones. However, Shibayama[123] could not confirm this fact.

In my experiments in agglutination of pest bacilli, the strain "Pest Avirulent" was agglutinated more easily than "Pest Virulent," but as pseudo-agglutinations occurred so frequently with the former strain, I am not willing to draw any conclusions from my experiments in regard to this question.

The pest bacillus is not a very favorable organism with which to study the relation between virulence and immunizing power, and a particularly unfavorable one with which to undertake binding experiments of the amboceptors in an immune serum. For this reason, I shall only briefly discuss the results which were obtained in immunization with several of the strains of different virulence. As may be seen from a number of the tables of inoculation, monkeys inoculated by thrusting subcutaneously a syringe needle which had been dipped in a suspension of the strain "Pest Virulent," frequently survived the inoculation and later showed themselves to be immune to pest infection. On the other hand, monkeys inoculated in the same manner, with a suspension of the strain "Pest Avirulent" always survived the inoculation, but later invariably showed no demonstrable immunity, and all succumbed to pest when reinoculated with a lethal dose of the virulent strain.

Throughout the numerous experiments performed in the vaccination of guinea pigs and monkeys with the strains "Pest Avirulent" and pest "Maassen Alt," it became clearly demonstrated that the latter organism was distinctly more virulent than the former. This fact may be confirmed from a study of the series of inoculated animals given in the tables, and also from them it may clearly be seen that the strain pest "Maassen Alt" produced a higher immunity in the animals which were inoculated with it than was brought about in those inoculated with the strain "Pest Avirulent." The following table summarizes these results:

Strain of plague bacillus.	Guinea pigs.			Monkeys.		
	Number inoculated with 1 agar slant culture.	Number died from inoculation.	Percentage found immune on subsequent test.	Number inoculated.	Number died from inoculation.	Percentage found immune on subsequent test.
"Pest Avirulent"	60	2	68.6	44	1	52
Pest "Maassen Alt"	47	6	88	49	5	70

[121] *Berl. Klin. Wchnsch.* (1902), **39**, 581.

[122] *Deutsche med. Wchnsch.* (1902), **28**, 45.

[123] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 482.

Therefore, my experiments would appear to confirm the earlier suggestions of R. Pfeiffer that the immunizing power of the plague organism is, within certain limits, proportional to its virulence. However, as my work relating to this question was performed with only three strains of the pest bacillus, it can not be considered to be conclusive for this organism in general and it can merely be stated that with the strains which I have studied, the more virulent organism gave rise to the greater immunity, and that the immunity within certain limits was proportional to the dose. (See experiments, pp. 199 to 221.)

XIV. RELATION OF THE IMMUNITY REACTIONS BETWEEN PEST, RINDERPEST, AND HÆMORRHAGIC SEPTICÆMIA.

The relationship which certain of the immunity reactions encountered in the study of pest and rinderpest bear to one another is sufficiently close to attract attention. Perhaps the greatest resemblance is seen in the action of the immune serum in the two diseases, which in each instance is anti-infectious in nature, possessing but little curative power. In rinderpest as in plague, there is little hope of saving the life of the animal by use of the serum, if the infection is well advanced before the serum is inoculated. The highest immunity in each of these diseases is also obtained by the inoculation of the living organism.

However, the plague bacillus presents a more uniform structure in the arrangement of its receptors than many of the other organisms of the hæmorrhagic septicæmia group. Thus, for example, it has been found in swine plague that a serum which may show high protective power for mice against several strains of bacilli of this group, against others will in these animals exert almost no beneficial action whatsoever. This is not true in the case of pest. A plague immune serum produced with one pest strain possesses polyvalent properties in that it will exert its anti-infectious action against all strains of the pest bacillus no matter what their source, and a plague polyvalent serum is not more or less effective in its action against any one of these different strains of pest than is a univalent one.

Kolle, Otto and Hetsch[124] found that guinea pigs or rats, only in very exceptional instances, could be immunized against pest infection by treatment with large doses of other living bacilli of the hæmorrhagic septicæmia group, and, vice versa, that animals immune to pest infection rarely showed any immunity against these pest-like bacteria.

From these experiments it seems established that the receptors of the two groups of bacteria are specific and differ considerably from one another and that the immunity is also specific in each instance and does not depend upon the more or less non-specific stimulation of the leucocytes, as Terni[125] has suggested in his most recent publication on this subject.

In connection with the experiments relating to the immunity reactions between pest bacilli and the organisms of the hæmorrhagic septicæmia group, it may be mentioned that we have noticed in Manila that cattle which had been rendered thoroughly immune to rinderpest were not immune to hæmorrhagic septicæmia and frequently succumbed from the latter disease. The immunity here is also in each instance specific. From all these reasons and particularly because apparently only ruminants are susceptible to rinderpest infection, it did not seem at all likely that guinea pigs which had been inoculated with virulent rinderpest blood or with antirinderpest serum would later show any immunity to pest

[124] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 364.
[125] *Ibid.* (1906), **54**, 385.

infection, but since in testing antirinderpest serum in the laboratory the opportunity presented itself of carrying on these experiments, they will here be recorded. Ten guinea pigs (see Series 66) were inoculated intraperitoneally each with 0.2 cubic centimeter of virulent rinderpest blood, 0.1 cubic centimeter of which, inoculated on the same day, gave rise to a fatal rinderpest infection in a steer. The guinea pigs showed no unfavorable symptoms following the inoculation. From one to three weeks later the immunity of the animals to plague infection was tested by rubbing a portion of a plague spleen from a guinea pig just dead of pest over a shaved area of the abdomen of the animals to be tested. None of the guinea pigs of the series showed any plague immunity whatever, as may be seen from the table, and all succumbed to acute plague infection. Ten other guinea pigs (Series 67) were each inoculated subcutaneously with 2 cubic centimeters of fresh antirinderpest serum. Six days later the immunity against plague of the animals was tested by the same method and all died of acute plague infection from the second to the fourth day after inoculation. Therefore, no relationship in immunity between these two infections could be stablished by these experiments, and they gave no encouragement for the performance of similar experiments on the larger and more expensive animals, such as cattle.

SERIES 66.—*Guinea pigs inoculated with virulent rinderpest blood, followed by plague infection.*

Animal No.	Inoculated January 19 with virulent rinderpest blood intraperitoneally.	Infected by shaving abdomen and rubbing with—	Result.	Remarks.
1708	0.2. cc.	Pest spleen of guinea pig No. 1702, Jan. 26.	Dead Jan. 29, after 3 days.	Culture from heart, pest bacilli.
1709	do	Pest spleen of guinea pig No. 1703, Jan. 27.	Dead Feb. 6, after 10 days.	Do.
1711	do	Pest spleen of guinea pig No. 1704, Jan. 28.	Dead Feb. 4, after 7 days.	Do.
1712	do	Pest spleen of guinea pig No. 1708, Jan. 29.	Dead Jan. 31, after 3 days.	Do.
1714	do	Pest spleen of guinea pig No. 1712, Jan. 31.	Dead Feb. 4, after 4 days.	Do.
1724	do	Pest spleen of guinea pig No. 1711, Feb. 4.	Dead Feb. 8, after 4 days.	Do.
1725	do	Pest spleen of guinea pig No 1714, Feb. 4.	Dead Feb. 7, after 3 days.	Do.
1728	do	Pest spleen of guinea pig No. 1709, Feb. 7.	Dead Feb. 10, after 3 days.	Do.
1729	do	Pest spleen of guinea pig No. 1725, Feb. 7.	Dead Feb. 12, after 5 days.	Do.
1745	do	Pest spleen of guinea pig No. 1724, Feb. 8.	Dead Feb. 12, after 4 days.	Do.

SERIES 67.

Each guinea pig of this series was inoculated subcutaneously with 2 cubic centimeters of fresh antirinderpest serum. Six days later each animal was reinoculated by massaging a shaved area over the abdomen with a portion of a spleen from a guinea pig (No. 3058) just dead of plague infection.

Guinea pig No.	Antirinderpestic serum subcutaneously.	Infected 6 days after injection of serum.	Result.	Remarks.
3074	2 cubic centimeters.	With pest	Dead after 2 days	Numerous pest bacilli in smears from spleen.
3075	do	do	Dead after 4 days	Do.
3076	do	do	Dead after 2 days	Do.
3077	do	do	do	Do.
3078	do	do	do	Do.
3079	do	do	Dead after 3 days	Do.
3080	do	do	do	Do.
3081	do	do	Dead after 4 days	Do.
3082	do	do	Dead after 2 days	Do.
3083	do	do	do	

XV. PLAGUE VACCINATION IN HUMAN BEINGS.

It having been conclusively demonstrated by animal experiments that by the method of vaccination against plague a much greater immunity could be produced than by any other method of inoculation, and earlier experiments [126] having demonstrated the entire safety of the inoculation of human beings with large amounts of the strain "Pest Avirulent," more extensive vaccinations were made in man with this culture. The size of the dose employed for an adult was always one 24-hour agar slant culture and for children from one-third to one-half this quantity. Surprising as it may seem, the injection of these large amounts of the living plague organism have not given rise to any very severe reactions. A few hours after the inoculation, the temperature of the individual usually begins to rise. When the injections have been made in the morning, the fever may in the evening of the first day reach from 38.5° to 39° C. Only in a few cases has it touched 40°. The temperature gradually declines on the following day and by the third of fourth one, has become normal. Occasionally, the cases showed a moderate leucocytosis after the injection. As in the earlier experiments, the organisms were always suspended in 1 cubic centimeter of 0.085 saline solution and the inoculations were made deeply into the deltoid muscle. Intramuscular instead of subcutaneous injections were performed on account of the quicker absorption which occurs from the former method, and because Meltzer and Auer [127] found that intramuscular injections as regards absorption in general stand in value very near that of a direct injection into the circulation. On the day after the vaccination there is usually distinct induration and redness about the point of the injection with some soreness on pressure, but these symptoms subside in from one to three days. No visible suppuration of the tissues has ever occurred.

In order to observe the length of time during which the organisms remained alive in the tissues, as mentioned, similar inoculations were made in monkeys and the tissues near the point of injection incised at varying intervals after the vaccination, and microscopical preparations and cultures made from the drops of blood which escaped from the wound. The technique employed was the same as that used in the experiments relating to increasing the virulence of the plague organism, as described on page 301. In the different series of cultures made from the animals

[126] *This Journal* (1906), I, 181.
[127] *Jour. Exp. Med.* (1905), 7, 77.

inoculated with the strain "Pest Avirulent" it was found that from six to eight hours after the time of the inoculation the organisms were still very numerous in the tissues, after which time they gradually diminished, and, usually after twenty-four hours, they were no longer reclaimable in cultures. However, in several instances a few colonies developed in the cultures made after this period of time. In immunized animals the organism was destroyed in a shorter period, the cultures made after twelve hours frequently remaining sterile. In these instances smears from the tissues three or four hours after the inoculation showed very extensive phagocytosis of the bacteria. Therefore, the process of immunization occurs as in a true vaccination, the organism reproducing itself in the tissues for probably 100 or 200 generations and its successive groups of receptors stimulating the production of corresponding groups of amboceptors in the animal body. It therefore is not difficult to understand why the immunity derived from vaccination in plague is greater than that obtained from the injection of the killed organism.

Although abundant evidence had been obtained of the immunity produced in animals even more susceptible to plague infection than man (guinea pigs) by vaccination with these attenuated cultures, yet it was interesting to observe what evidence could be discovered, from a study of the blood serum, of the immunity resulting in human beings from such vaccinations. Therefore, the agglutinative reaction of the blood serum of twenty-six of the cases and the anti-infectious power in twenty-four were carefully studied. In order that the experiments might be carefully and completely performed, from 10 to 20 cubic centimeters of blood was collected from a vein, in each case under aseptic precautions; the blood being drawn ten days after vaccination and the reactions usually performed as soon as the serum had separated from the clot. The results of these experiments have already been recorded during the consideration of the agglutinative and anti-infectious properties of plague serum. The agglutinative reactions are also summarized in Table No. X (p. 252), and the anti-infectious ones in the series of inoculations on pages 275 to 280. From these tables it will be seen that the sera possess practically no agglutinating or anti-infectious power, at least in demonstrable amounts. Only in a few instances were there traces of a reaction. However, since in the serum of animals which had been demonstrated to be thoroughly immune to plague these substances (agglutinins and anti-infectious bodies) could not be demonstrated, we should hardly expect to find them in the blood serum of vaccinated human beings. Plague agglutinins and anti-infectious bodies only become developed in demonstrable amounts in animals that have been very highly immunized by large and repeated inoculations of the organism, as has already conclusively been demonstrated.

The opsonic power of the sera of the vaccinated individuals was next carefully investigated in eight instances, and this reaction proved to

be a much more delicate and accurate means of demonstrating the existence of an immunity reaction following plague vaccination, than those already described. The experiments were performed with the strain "Pest Virulent" in the following manner:

The blood was collected 10 days after vaccination. 0.1 cubic centimeter of the serum, 0.1 cubic centimeter of a suspension of a normal guinea pig's washed leucocytes and 0.1 cubic centimeter of a suspension of the strain "Pest Virulent" were thoroughly mixed and the resulting suspension placed for thirty minutes at 37° C. Smears were then prepared and stained and 200 leucocytes counted. Control experiments of the nonphagocytic power of the washed leucocytes without serum were always prepared.

Series 62 (p. 267) shows the opsonic index both before and after vaccination. No attempt was made by frequent examination, to demonstrate a negative phase immediately following vaccination or to plot the curve of the reaction. As the table demonstrates, there was usually a distinct increase in the opsonic index following the inoculation, but this was not invariably the case. The increase in the opsonic action of the blood in animals immunized against plague has already been discussed under the subject of the immunizing action of plague immune sera. (See p. 265.)

The most satisfactory test of the demonstration of the immunity reaction in the blood of the vaccinated individuals was obtained from a study of the action of the serum in relation to the phenomenon of fixation of the complement after the method of Bordet and Gengou. These experiments have already been referred to under the discussion of the mechanism of the action of plague immune serum. Hæmolytic sera were first prepared by inoculating rabbits with goats' corpuscles; these were then collected from the rabbits, inactivated, and their hæmolytic power for a 5 per cent suspension of serum-free goat's corpuscles carefully determined. Fresh guinea pig serum was used to furnish the complement, and the minimum amount of complement for the reaction also ascertained. The human serum to be tested for plague immune bodies was then inactivated by heating for one hour at 56° C. A suspension in saline solution of a 24-hour agar culture of the virulent pest strain which was free of all clumps was employed. The experiments were performed in the following manner:

One cubic centimeter of the diluted human inactivated serum from the vaccinated person was added to 1 cubic centimeter of the suspension of bacteria and the unit dose of complement mixed with it. After one hour at 37° C., twice the dissolving dose of inactivated rabbits' serum specifically hæmolytic for goats' corpuscles and suspended in 1 cubic centimeter saline solution and 1 cubic centimeter of the 5 per cent solution of goats' corpuscles was added and the mixtures placed at 37° C. for 2 hours. At the end of this time the reactions were

noted. All the necessary controls were also performed as may be seen from the tables where all the details of the reactions are given. The experiments were performed with the sera of four of the human beings and of two guinea pigs which had been vaccinated and with the serum of one plague immune horse. In each of these instances the deflection of the complement was obtained, demonstrating the presence of plague immune bodies in the serum tested. The results obtained are summarized in the following tables.

TABLE No. XI.—*Showing phenomenon of fixation of the complement with the serum of human beings and animals vaccinated against plague.*

[Saline suspension 0.85 per cent, q. s. to 4 cubic centimeters in each test tube.]

Inactivated rabbits' serum hæmolytic for goats' corpuscles.	5 per cent suspension goats' corpuscles.	Guinea pig's fresh serum for complement.	Vaccinated guinea pig serum (inactivated).		Vaccinated human serum No. I (inactivated).	Normal guinea pig serum (inactivated).	Normal human serum (inactivated).	Saline suspension of strain "Pest Virulent."	Hæmolysis.
			No. I.	No. II.					
0.0025	1	0.2							+
.005	1	.2							+
.005	1	.2						1	+
.005	1	.2	0.1						+
.005	1	.2	.025						+
.005	1	.2		0.05					+
.005	1	.2		.025					+
.005	1	.2			0.1				+
.005	1	.2	.1					1	0
.005	1	.2	.025					1	0
.005	1	.2		.05				1	0
.005	1	.2		.025				1	0
.005	1	.2			.1			1	0
.005	1	.2				0.25		1	+
.005	1	.2				.1		1	+
.005	1	.2				.025		1	+
.005	1	.2					0.25	1	+
.005	1	.2					.1	1	+
.005	1	.2				.25			+
.005	1	.2				.1			+
.005	1	.2				.025			+
.005	1	.2					.25		+
.005	1	.2					.1		+
.005	1		.1					1	0
.005	1		.025					1	0
.005	1			.05				1	0
.005	1			.025				1	0
.005	1				.1			1	0
.005	1							1	0
	1	.2							0
.005	1								0
	1								0

TABLE No. XII.—*Showing phenomenon of fixation of the complement with the serum of human beings vaccinated against plague and with horses' pest immune serum.*

[Saline suspension 0.85 per cent, q. s. to 4 cubic centimeters in each test tube.]

Inactivated rabbits' serum hæmolytic for goats' corpuscles.	5 per cent suspension goats' corpuscles.	Guinea pig's fresh serum for complement.	Horses' pest immune serum (inactivated).	Vaccinated human serum (inactivated).			Normal horses' serum (inactivated).	Normal human serum (inactivated).	Saline suspension of strain "Pest Virulent."	Hæmolysis.
				No. II.	No. III.	No. IV.				
0.0016	1	0.15								+
.0033	1	.15								±
.0033	1	.15							1	+
.0033	1	.15	0.02							+
.0033	1	.15	.01							+
.0033	1	.15		0.1						+
.0033	1	.15			0.1					+
.0033	1	.15			.05					+
.0033	1	.15				0.05				+
.0033	1	.15				.033				+
.0033	1	.15	.02						1	0
.0033	1	.15	.01						1	0
.0033	1	.15		.1					1	0
.0033	1	.15			.1				1	0
.0033	1	.15			.05				1	0
.0033	1	.15				.05			1	0
.0033	1	.15				.033			1	0
.0033	1	.15					0.05		1	+
.0033	1	.15					.01		1	+
.0033	1	.15						0.1	1	+
.0033	1	.15						.05	1	+
.0033	1	.15					.05			+
.0033	1	.15					.01			+
.0033	1	.15						.1		+
.0033	1	.15						.05		+
.0033	1		.02						1	0
.0033	1		.01						1	0
.0033	1			.1					1	0
.0033	1				.1				1	0
.0033	1				.05				1	0
.0033	1					.05			1	0
.0033	1					.033			1	0
.0033	1								1	0
	1	.15								0
.0033	1									0
	1									0

Therefore, following plague vaccination in human beings, an immunity reaction occurs which consists in the development of anti-bodies thrown off into the circulation and whose presence may be demonstrated both by the opsonic reaction of Wright and also by the method of the fixation of the complement of Bordet and Gengou.

Since my first report upon the method of vaccination against plague in man in 1905, such inoculations have been made from time to time

and during the past year nearly 200 vaccinations have been performed. The cultures with which I have worked have proven themselves to be entirely safe for human beings [128] and I have observed no unfavorable results in the inoculated. However, as emphasized in my previous communication, vaccinations against plague should not be made in man unless the investigator can guarantee the particular organism with which he is working to be of sufficient attenuation to be no longer dangerous for human beings. Strains of the bacilli which invariably no longer kill guinea pigs of 250 grams weight upon subcutaneous inoculation in amounts of one agar slant culture are probably safe in small amounts for human beings. Owing to the great stability in virulence of the plague bacillus under certain conditions, its use in vaccination is much simplified.

During the past year and a half I have been able to detect no change either in virulence, toxicity, or immunizing power in the strains with which I have performed my vaccinations in man and in animals. The cultures apparently possess the same immunizing power to-day as they did at the commencement of the experiments. However, it is very probable that they could be further attenuated by the artificial means previously described.

Douglas and W. Bullock [129] (whose article on plague has just appeared in Allbutt and Rolleston's System of Medicine as my article goes to press) in commenting upon my work in vaccination against plague make the following statement:

> Naturally very great care would be necessary in recommending a method like this on a big scale in plague stricken communities, as from unforeseen circumstances the virulence might increase and plague be induced.

As I have pointed out, there is no evidence to support this statement, and my cultures which for nearly two years have been used at intervals in human beings are as safe for use in man to-day as they were at the time of my first inoculations.

[128] The accident in relation to some inoculations which recently occurred in Bilibid Prison in Manila and about which a report will shortly be made, was not due to these plague vaccinations.

[129] Allbutt and Rolleston: System of Medicine (1907), **2**, part 2, 408.

XVI. CONCLUSION.

In recommending for man vaccination against plague with suitable cultures I wish to emphasize the fact that the method is not infallible and that very brilliant results may not always be obtained by it. In the vaccination of large numbers of human beings, owing to individual variations in susceptibility to plague infection and natural resistance, just as in the experiments described in monkeys, certainly all of the individuals will not be protected against lethal infection by a single vaccination with one agar culture, but a certain proportion of them may be immunized by this method and an appreciable degree of immunity may be retained for at least nine months. The population in a plague stricken district may gradually be immunized against this disease by the employment of vaccination.

In plague, as in smallpox vaccinations, it may frequently be necessary to repeat the vaccination and perhaps with a larger dose, in order to secure a satisfactory immunity.

As yet we know of no practical method for the detection of those cases which would require a second plague vaccination to produce in them a sufficient immunity to protect them from the natural manner of infection of the disease. It is perhaps possible that by a careful study of the opsonic index in man and animals both before and after vaccination and with subsequent attempts to infect the animal in order to observe the degree of immunization which has resulted, a standard may be obtained by means of which we may be able to judge from the amount of the increase in the index whether the individual has acquired a satisfactory immunity from the first vaccination.

Owing to the complexity of the experiments necessary for the determination of the phenomenon of the fixation of the complement in the serum of the vaccinated, this method will probably never assume practical value for the determination in a large number of cases of the degree of plague immunity acquired.

Professor Kitasato in a recent address published in a previous number of this JOURNAL [130] made the statement that:

> Plague is not only objectionable to the people of a locality but it is an enemy to mankind in general. All civilized nations must fight this common enemy. I believe there should be an international conference to discuss a plan to collect money and to organize an international army to combat and vanquish this disease wherever it appears. Expeditions should be sent to the interior regions

[130] *This Journal* (1906), 1, 465.

of India and South China. The cost of such an enterprise would be only a small part of what the civilized nations are constantly expending in keeping armies and navies. Even the amount which every country is spending for the prevention of the pestilence would suffice. My suggestion only lacks a leader, and I see that the United States, one of the greatest nations of the earth, has such a leader in the person of its president, Theodore Roosevelt, who has already done so much for humanity and whose noble works are being admired by the whole world.

It is obvious that sufficient attention is not being given to combating this disease in its endemic centers and from which there is continued danger of invasion by the pestilence into other countries. This fact is conclusively demonstrated because the disease is not markedly decreasing in these centers. Indeed it would appear that India is at present suffering from an epidemic of plague equal to the one which occurred there in 1904. The official monthly returns from that country for the present year (1907) show 58,438 deaths from plague in January, 98,397 in February and 171,522 in March.[131]

During the past year the British Indian Plague Commission, under the direction of Martin and Lamb, have by their very important studies thrown much light upon the question of the manner of transmission of the disease. Frequent reference has been made in the present article to their researches as well as to the very valuable ones of Kolle and his pupils in Berlin, and it is hoped that the laboratory investigations of these observers together with the studies of Kitasato in Japan will serve further to stimulate more active work in the extermination of this pestilence.

From my investigations it would appear that general vaccination in the endemic centers will be a valuable means of assistance in accomplishing this end.

[131] *Lancet.* (1907), 1, 1200.